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(54) Title: USES OF AGONISTS AND ANTAGONISTS TO MODULATE ACTIVITY OF TNF-RELATED MOLECULES

(57) Abstract: Methods of using one or more agonists or antagonists to modulate activity of the members of the TNF and TNFR families referred to as TALL-1, APRIL, TACI, and BCMA are provided. The methods include in vitro, in situ, and/or in vivo diagnosis and/or treatment of mammalian cells or pathological conditions associated with TALL-1, APRIL, TACI, or BCMA, using one or more agonist or antagonist molecules. The methods of treatment disclosed by the invention include methods of treating immune related diseases and cancer.

Uses of Agonists and Antagonists to Modulate Activity of
TNF-Related Molecules

FIELD OF THE INVENTION

5   This invention relates generally to methods of using one or more
agonists or antagonists to modulate activity of tumor necrosis factor
(TNF) and TNF receptor (TNFR)-related molecules, and more
specifically, the members of the TNF and TNFR families referred to as
TALL-1, APRIL, TACI, and BCMA. The invention also relates to methods

10   for *in vitro, in situ,* and/or *in vivo* diagnosis and/or treatment of
mammalian cells or pathological conditions associated with TALL-1,
APRIL, TACI, or BCMA, using one or more agonist or antagonist
molecules.

BACKGROUND OF THE INVENTION

15   Various molecules, such as tumor necrosis factor-α ("TNF-α"),
tumor necrosis factor-β ("TNF-β" or "lymphotoxin-α"), lymphotoxin-β
("LT-β"), CD30 ligand, CD27 ligand, CD40 ligand, OX-40 ligand, 4-1BB
ligand, Apo-1 ligand (also referred to as Fas ligand or CD95 ligand),

20   Apo-2 ligand (also referred to as TRAIL), Apo-3 ligand (also referred
to as TWEAK), osteoprotegerin (OPG), APRIL, RANK ligand (also
referred to as TRANCE), and TALL-1 (also referred to as BlyS, BAFF or
THANK) have been identified as members of the tumor necrosis factor
("TNF") family of cytokines [See, e.g., Gruss and Dower, Blood,

25   85:3378-3404 (1995); Pitti et al., J. Biol. Chem., 271:12687-12690
(1996); Wiley et al., Immunity, 3:673-682 (1995); Browning et al.,
Cell, 72:847-856 (1993); Armitage et al. Nature, 357:80-82 (1992), WO
97/01633 published January 16, 1997; WO 97/25428 published July 17,
1997; Marsters et al., Curr. Biol., 8:525-528 (1998); Simonet et al.,

30   Cell, 89:309-319 (1997); Chicheportiche et al., Biol. Chem.,
272:32401-32410 (1997); Hahne et al., J. Exp. Med., 188:1185-1190
(1998); WO98/28426 published July 2, 1998; WO98/46751 published
October 22, 1998; WO/98/18921 published May 7, 1998; Moore et al.,
Science, 285:260-263 (1999); Shu et al., J. Leukocyte Biol., 65:680

35   (1999); Schneider et al., J. Exp. Med., 189:1747-1756 (1999);
Mukhopadhyay et al., J. Biol. Chem., 274:15978-15981 (1999)]. Among
these molecules, TNF-α, TNF-β, CD30 ligand, 4-1BB ligand, Apo-1
ligand, Apo-2 ligand (Apo2L/TRAIL) and Apo-3 ligand (TWEAK) have been
reported to be involved in apoptotic cell death. Both TNF-α and TNF-

β have been reported to induce apoptotic death in susceptible tumor cells [Schmid et al., Proc. Natl. Acad. Sci., 83:1881 (1986); Dealtry et al., Eur. J. Immunol., 17:689 (1987)].

Various molecules in the TNF family also have purported role(s) in the function or development of the immune system [Gruss et al., Blood, 85:3378 (1995)]. Zheng et al. have reported that TNF-α is involved in post-stimulation apoptosis of CD8-positive T cells [Zheng et al., Nature, 377:348-351 (1995)]. Other investigators have reported that CD30 ligand may be involved in deletion of self-reactive T cells in the thymus [Amakawa et al., Cold Spring Harbor Laboratory Symposium on Programmed Cell Death, Abstr. No. 10, (1995)]. CD40 ligand activates many functions of B cells, including proliferation, immunoglobulin secretion, and survival [Renshaw et al., J. Exp. Med., 180:1889 (1994)]. Another recently identified TNF family cytokine, TALL-1 (BlyS), has been reported, under certain conditions, to induce B cell proliferation and immunoglobulin secretion. [Moore et al., supra; Schneider et al., supra; Mackay et al., J. Exp. Med., 190:1697 (1999)].

Mutations in the mouse Fas/Apo-1 receptor or ligand genes (called lpr and gld, respectively) have been associated with some autoimmune disorders, indicating that Apo-1 ligand may play a role in regulating the clonal deletion of self-reactive lymphocytes in the periphery [Krammer et al., Curr. Op. Immunol., 6:279-289 (1994); Nagata et al., Science, 267:1449-1456 (1995)]. Apo-1 ligand is also reported to induce post-stimulation apoptosis in CD4-positive T lymphocytes and in B lymphocytes, and may be involved in the elimination of activated lymphocytes when their function is no longer needed [Krammer et al., supra; Nagata et al., supra]. Agonist mouse monoclonal antibodies specifically binding to the Apo-1 receptor have been reported to exhibit cell killing activity that is comparable to or similar to that of TNF-α [Yonehara et al., J. Exp. Med., 169:1747-1756 (1989)].

Induction of various cellular responses mediated by such TNF family cytokines is believed to be initiated by their binding to specific cell receptors. Previously, two distinct TNF receptors of approximately 55-kDa (TNFR1) and 75-kDa (TNFR2) were identified [Hohman et al., J. Biol. Chem., 264:14927-14934 (1989); Brockhaus et al., Proc. Natl. Acad. Sci., 87:3127-3131 (1990); EP 417,563, published March 20, 1991; Loetscher et al., Cell, 61:351 (1990);

Schall et al., Cell, 61:361 (1990); Smith et al., Science, 248:1019-
1023 (1990); Lewis et al., Proc. Natl. Acad. Sci., 88:2830-2834
(1991); Goodwin et al., Mol. Cell. Biol., 11:3020-3026 (1991)].
Those TNFRs were found to share the typical structure of cell surface

5   receptors including extracellular, transmembrane and intracellular
regions. The extracellular portions of both receptors were found
naturally also as soluble TNF-binding proteins [Nophar, Y. et al.,
EMBO J., 9:3269 (1990); and Kohno, T. et al., Proc. Natl. Acad. Sci.
U.S.A., 87:8331 (1990); Hale et al., J. Cell. Biochem. Supplement

10 15F, 1991, p. 113 (P424)].

        The extracellular portion of type 1 and type 2 TNFRs (TNFR1 and
TNFR2) contains a repetitive amino acid sequence pattern of four
cysteine-rich domains (CRDs) designated 1 through 4, starting from
the NH_2-terminus. [Schall et al., supra; Loetscher et al., supra;

15 Smith et al., supra; Nophar et al., supra; Kohno et al., supra;
Banner et al., Cell, 73:431-435 (1993)]. A similar repetitive
pattern of CRDs exists in several other cell-surface proteins,
including the p75 nerve growth factor receptor (NGFR) [Johnson et
al., Cell, 47:545 (1986); Radeke et al., Nature, 325:593 (1987)], the

20 B cell antigen CD40 [Stamenkovic et al., EMBO J., 8:1403 (1989)], the
T cell antigen OX40 [Mallet et al., EMBO J., 9:1063 (1990)] and the
Fas antigen [Yonehara et al., supra and Itoh et al., Cell, 66:233-243
(1991)]. CRDs are also found in the soluble TNFR (sTNFR)-like T2
proteins of the Shope and myxoma poxviruses [Upton et al., Virology,

25 160:20-29 (1987); Smith et al., Biochem. Biophys. Res. Commun.,
176:335 (1991); Upton et al., Virology, 184:370 (1991)]. Optimal
alignment of these sequences indicates that the positions of the
cysteine residues are well conserved. These receptors are sometimes
collectively referred to as members of the TNF/NGF receptor

30 superfamily.

        The TNF family ligands identified to date, with the exception of
lymphotoxin-α, are type II transmembrane proteins, whose C-terminus
is extracellular. In contrast, most receptors in the TNF receptor
(TNFR) family identified to date are type I transmembrane proteins.

35 In both the TNF ligand and receptor families, however, homology
identified between family members has been found mainly in the
extracellular domain ("ECD"). Several of the TNF family cytokines,
including TNF-α, Apo-1 ligand and CD40 ligand, are cleaved
proteolytically at the cell surface; the resulting protein in each

case typically forms a homotrimeric molecule that functions as a soluble cytokine. TNF receptor family proteins are also usually cleaved proteolytically to release soluble receptor ECDs that can function as inhibitors of the cognate cytokines.

5   More recently, other members of the TNFR family have been identified. In von Bulow et al., Science, 278:138-141 (1997), investigators describe a plasma membrane receptor referred to as Transmembrane Activator and CAML-Interactor or "TACI". The TACI receptor is reported to contain a cysteine-rich motif characteristic
10   of the TNFR family and to be present on both B cells and activated T cells. In an *in vitro* assay, cross linking of TACI on the surface of transfected Jurkat cells with TACI-specific antibodies led to activation of NF-KB. [see also, WO 98/39361 published September 18, 1998].

15   Laabi et al., EMBO J., 11:3897-3904 (1992) reported identifying a new gene called "BCM" whose expression was found to coincide with B cell terminal maturation. The open reading frame of the BCM normal cDNA predicted a 184 amino acid long polypeptide with a single transmembrane domain. These investigators later termed this gene
20   "BCMA." [Laabi et al., Nucleic Acids Res., 22:1147-1154 (1994)]. BCMA mRNA expression was reported to be absent in human malignant B cell lines which represent the pro-B lymphocyte stage, and thus, is believed to be linked to the stage of differentiation of lymphocytes [Gras et al., Int. Immunology, 7:1093-1106 (1995)]. In Madry et al.,
25   Int. Immunology, 10:1693-1702 (1998), the cloning of murine BCMA cDNA was described. The murine BCMA cDNA is reported to encode a 185 amino acid long polypeptide having 62% identity to the human BCMA polypeptide. Alignment of the murine and human BCMA protein sequences revealed a conserved motif of six cysteines in the N-
30   terminal region, suggesting that the BCMA protein belongs to the TNFR superfamily [Madry et al., supra].

In Marsters et al., Curr. Biol., 6:750 (1996), investigators describe a full length native sequence human polypeptide, called Apo-3, which exhibits similarity to the TNFR family in its extracellular
35   cysteine-rich repeats and resembles TNFR1 and CD95 in that it contains a cytoplasmic death domain sequence [see also Marsters et al., Curr. Biol., 6:1669 (1996)]. Apo-3 has also been referred to by other investigators as DR3, wsl-1, TRAMP, and LARD [Chinnaiyan et al., Science, 274:990 (1996); Kitson et al., Nature, 384:372 (1996);

Bodmer et al., Immunity, 6:79 (1997); Screaton et al., Proc. Natl.
Acad. Sci., 94:4615-4619 (1997)].

Pan et al. have disclosed another TNF receptor family member
referred to as "DR4" [Pan et al., Science, 276:111-113 (1997); see
5   also WO98/32856 published July 30, 1998]. The DR4 was reported to
contain a cytoplasmic death domain capable of engaging the cell
suicide apparatus. Pan et al. disclose that DR4 is believed to be a
receptor for the ligand known as Apo2L/TRAIL.

In Sheridan et al., Science, 277:818-821 (1997) and Pan et al.,
10   Science, 277:815-818 (1997), another molecule believed to be a
receptor for Apo2L/TRAIL is described [see also, WO98/51793 published
November 19, 1998; WO98/41629 published September 24, 1998]. That
molecule is referred to as DR5 (it has also been alternatively
referred to as Apo-2; TRAIL-R, TR6, Tango-63, hAPO8, TRICK2 or KILLER
15   [Screaton et al., Curr. Biol., 7:693-696 (1997); Walczak et al., EMBO
J., 16:5386-5387 (1997); Wu et al., Nature Genetics, 17:141-143
(1997); WO98/35986 published August 20, 1998; EP 870,827 published
October 14, 1998; WO98/46643 published October 22, 1998; WO99/02653
published January 21, 1999; WO99/09165 published February 25, 1999;
20   WO99/11791 published March 11, 1999]. Like DR4, DR5 is reported to
contain a cytoplasmic death domain and be capable of signaling
apoptosis. The crystal structure of the complex formed between Apo-
2L/TRAIL and DR5 is described in Hymowitz et al., Molecular Cell,
4:563-571 (1999).

25   Yet another death domain-containing receptor, DR6, was recently
identified [Pan et al., FEBS Letters, 431:351-356 (1998)]. Aside from
containing four putative extracellular cysteine rich domains and a
cytoplasmic death domain, DR6 is believed to contain a putative
leucine-zipper sequence that overlaps with a proline-rich motif in
30   the cytoplasmic region. The proline-rich motif resembles sequences
that bind to src-homology-3 domains, which are found in many
intracellular signal-transducing molecules.

A further group of recently identified receptors are referred to
as "decoy receptors," which are believed to function as inhibitors,
35   rather than transducers of signaling. This group includes DCR1 (also
referred to as TRID, LIT or TRAIL-R3) [Pan et al., Science, 276:111-
113 (1997); Sheridan et al., Science, 277:818-821 (1997); McFarlane
et al., J. Biol. Chem., 272:25417-25420 (1997); Schneider et al.,
FEBS Letters, 416:329-334 (1997); Degli-Esposti et al., J. Exp. Med.,
40   186:1165-1170 (1997); and Mongkolsapaya et al., J. Immunol., 160:3-6

(1998)] and DCR2 (also called TRUNDD or TRAIL-R4) [Marsters et al., Curr. Biol., 7:1003-1006 (1997); Pan et al., FEBS Letters, 424:41-45 (1998); Degli-Esposti et al., Immunity, 7:813-820 (1997)], both cell surface molecules, as well as OPG [Simonet et al., supra; Emery et

5 al., infra] and DCR3 [Pitti et al., Nature, 396:699-703 (1998)], both of which are secreted, soluble proteins.

Additional newly identified members of the TNFR family include CAR1, HVEM, GITR, ZTNFR-5, NTR-1, and TNFL1 [Brojatsch et al., Cell, 87:845-855 (1996); Montgomery et al., Cell, 87:427-436 (1996);

10 Marsters et al., J. Biol. Chem., 272:14029-14032 (1997); Nocentini et al., Proc. Natl. Acad. Sci. USA 94:6216-6221 (1997); Emery et al., J. Biol. Chem., 273:14363-14367 (1998); WO99/04001 published January 28, 1999; WO99/07738 published February 18, 1999; WO99/33980 published July 8, 1999].

15 As reviewed recently by Tewari et al., TNFR1, TNFR2 and CD40 modulate the expression of proinflammatory and costimulatory cytokines, cytokine receptors, and cell adhesion molecules through activation of the transcription factor, NF-κB [Tewari et al., Curr. Op. Genet. Develop., 6:39-44 (1996)]. NF-κB is the prototype of a

20 family of dimeric transcription factors whose subunits contain conserved Rel regions [Verma et al., Genes Develop., 9:2723-2735 (1996); Baldwin, Ann. Rev. Immunol., 14:649-681 (1996)]. In its latent form, NF-κB is complexed with members of the IκB inhibitor family; upon inactivation of the IκB in response to certain stimuli,

25 released NF-κB translocates to the nucleus where it binds to specific DNA sequences and activates gene transcription. As described above, the TNFR members identified to date either include or lack an intracellular death domain region. Some TNFR molecules lacking a death domain, such as TNFR2, CD40, HVEM, and GITR, are capable of

30 modulating NF-κB activity. [see, e.g., Lotz et al., J. Leukocyte Biol., 60:1-7 (1996)].

For a review of the TNF family of cytokines and their receptors, see Ashkenazi and Dixit, Science, 281:1305-1308 (1998); Golstein, Curr. Biol., 7:750-753 (1997); Gruss and Dower, supra, and Nagata,

35 Cell, 88:355-365 (1997).

SUMMARY OF THE INVENTION

Applicants have surprisingly found that the TNF family ligand referred to as TALL-1 binds to the TACI receptor and to the BCMA

receptor. Applicants have also surprisingly found that the TNF family ligand referred to as APRIL binds to both the TACI and BCMA receptors. Although certain TALL-1 and APRIL ligands, and certain TACI and BCMA receptors, have been described previously, it was not appreciated in the art that TALL-1 and APRIL bind and activate the TACI and BCMA receptors. The present invention thus provides for novel methods of using antagonists or agonists of these TNF-related ligands and receptors. The antagonists and agonists described herein find utility for, among other things, *in vitro*, *in situ*, or *in vivo* diagnosis or treatment of mammalian cells or pathological conditions associated with the presence (or absence) of TALL-1, APRIL, TACI, or BCMA.

The methods of use include methods to treat pathological conditions or diseases in mammals associated with or resulting from increased or enhanced TALL-1 or APRIL expression and/or activity. In the methods of treatment, TALL-1 antagonists or APRIL antagonists may be administered to the mammal suffering from such pathological condition or disease. The TALL-1 antagonists and APRIL antagonists contemplated for use in the invention include TACI receptor immunoadhesins or BCMA receptor immunoadhesins, as well as antibodies against the TACI receptor or BCMA receptor, which preferably block or reduce the respective receptor binding or activation by TALL-1 ligand or APRIL ligand. For instance, TACI receptor immunoadhesins may be employed to treat rheumatoid arthritis or multiple sclerosis. The TALL-1 antagonists and APRIL antagonists contemplated for use further include anti-TALL-1 antibodies or anti-APRIL antibodies which are capable of blocking or reducing binding of the respective ligands to the TACI or BCMA receptors. Still further antagonist molecules include covalently modified forms, or fusion proteins, comprising TACI or BCMA. By way of example, such antagonists may include pegylated TACI or BCMA and TACI or BCMA fused to heterologous sequences such as epitope tags or leucine zippers. Optionally, the antagonist molecule(s) employed in the methods will be capable of blocking or neutralizing the activity of both TALL-1 and APRIL, e.g., a dual antagonist which blocks or neutralizes activity of both TALL-1 and APRIL. The methods contemplate the use of a single type of antagonist molecule or a combination of two or more types of antagonist.

In another embodiment of the invention, there are provided methods for the use of TALL-1 antagonists to block or neutralize the

7

interaction between TALL-1 and TACI and/or BCMA. For example, the invention provides a method comprising exposing a mammalian cell, such as a white blood cell (preferably a B cell), to one or more TALL-1 antagonists in an amount effective to decrease, neutralize or

5   block activity of the TALL-1 ligand. The cell may be in cell culture or in a mammal, e.g. a mammal suffering from, for instance, an immune related disease or cancer. Thus, the invention includes a method for treating a mammal suffering from a pathological condition such as an immune related disease or cancer comprising administering an

10   effective amount of one or more TALL-1 antagonists, as disclosed herein.

    The invention also provides methods for the use of APRIL antagonists to block or neutralize the interaction between APRIL and TACI or BCMA. For example, the invention provides a method

15   comprising exposing a mammalian cell, such as a white blood cell (preferably a B cell), to one or more APRIL antagonists in an amount effective to decrease, neutralize or block activity of the APRIL ligand. The cell may be in cell culture or in a mammal, e.g. a mammal suffering from, for instance, an immune related disease or

20   cancer. Thus, the invention includes a method for treating a mammal suffering from a pathological condition such as an immune related disease or cancer comprising administering an effective amount of one or more APRIL antagonists, as disclosed herein.

    The invention also provides compositions which comprise one or

25   more TALL-1 antagonists or APRIL antagonists. Optionally, the compositions of the invention will include pharmaceutically acceptable carriers or diluents. Preferably, the compositions will include one or more TALL-1 antagonists or APRIL antagonists in an amount which is therapeutically effective to treat a pathological

30   condition or disease.

    The invention also provides particular antagonist molecules comprising anti-APRIL antibodies. Such antibodies may include monoclonal antibodies, chimeric antibodies, humanized antibodies or human antibodies. In one embodiment, the anti-APRIL antibodies

35   comprise monoclonal antibodies, and preferably, comprise the anti-APRIL monoclonal antibodies disclosed in the Examples below. Hybridomas secreting various anti-APRIL monoclonal antibodies are further provided herein.

    The invention also provides articles of manufacture and kits

40   which include one or more TALL-1 antagonists or APRIL antagonists.

                                    8

In addition, the invention provides methods of using TACI
agonists or BCMA agonists to, for instance, stimulate or activate
TACI receptor or BCMA receptor. Such methods will be useful in
treating pathological conditions characterized by or associated with
5   insufficient TALL-1 or APRIL expression or activity such as
immunodeficiency or cancer (such as by boosting the immune anti-
cancer response). The TACI agonists or BCMA agonists may comprise
agonistic anti-TACI or anti-BCMA antibodies. The agonistic activity
of such TACI agonists or BCMA agonists may comprise enhancing the
10 activity of a native ligand for TACI or BCMA or activity which is the
same as or substantially the same as (i.e., mimics) the activity of a
native ligand for TACI or BCMA.

Thus, the invention also provides compositions which comprise
one or more TACI agonists or BCMA agonists. Optionally, the
15 compositions of the invention will include pharmaceutically
acceptable carriers or diluents. Preferably, the compositions will
include one or more TACI agonists or BCMA agonists in an amount which
is therapeutically effective to stimulate signal transduction by TACI
or BCMA.

20 Further, the invention provides articles of manufacture and kits
which include one or more TACI agonists or BCMA agonists.

The invention also provides methods of conducting screening
assays to identify candidate molecules, such as small molecule
compounds, polypeptides or antibodies, which act as agonists or
25 antagonists with respect to the interaction between TALL-1 and TACI
or BCMA, or to the interaction between APRIL and TACI or BCMA.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1B show a polynucleotide sequence encoding a native
30 sequence human TACI (SEQ ID NO:1) (reverse complimentary sequence is
provided in SEQ ID NO:11) and its putative amino acid sequence (SEQ
ID NO:2).

Figure 2 shows a polynucleotide sequence encoding a native
35 sequence human BCMA (SEQ ID NO:3) (reverse complimentary sequence is
provided in SEQ ID NO:12) and its putative amino acid sequence (SEQ
ID NO:4).

Figure 3 shows a polynucleotide sequence encoding a native
40 sequence human TALL-1 (SEQ ID NO:5) (reverse complimentary sequence

is provided in SEQ ID NO:13) and its putative amino acid sequence
(SEQ ID NO:6).

Figures 4A-4B show a polynucleotide sequence encoding a native
5   sequence human APRIL (SEQ ID NO:7) (reverse complimentary sequence is
provided in SEQ ID NO:14) and its putative amino acid sequence (SEQ
ID NO:8).

Figures 5A-5B show exemplary methods for calculating the % amino
10 acid sequence identity of the amino acid sequence designated
"Comparison Protein" to the amino acid sequence designated "PRO". For
purposes herein, the "PRO" sequence may be the TACI, BCMA, TALL-1, or
APRIL sequences referred to in Figures 1, 2, 3, or 4 herein,
respectively.
15

Figure 6 shows an alignment of two amino acid sequences for the
TACI receptor, referred to as "hTACI (265)" (SEQ ID NO:9), believed
to be a spliced variant, and "hTACI", also referred to in Figures 1A-
1B (SEQ ID NO:2).
20

Figures 7A-7D show the results of an *in situ* assay, staining for
AP activity. COS 7 cells were transfected with TACI (Fig. 7A-7C) or
vector plasmid (Fig. 7D) and incubated with AP-TALL-1 (Fig. 7A, 7D);
AP-TNF-alpha (Fig. 7B); or AP-EDA (Fig. 7C).
25

Figures 8A-8H show the results, by Western blot analysis, of
various co-immunoprecipitation assays of TALL-1 or APRIL with TACI or
BCMA immunoadhesins, as described in detail in Example 2. In Figures
8A-8H, TALL-1 is referred to as "Blys/TALL-1".
30

Figures 9A-9B show the results of further binding assays to
demonstrate TALL-1 and APRIL are ligands for TACI and BCMA. In
Figure 9A, COS 7 cells were transfected with TALL-1 (9a-9c); APRIL
(9d-9f); or TNF-alpha (9g-9i) and incubated with BCMA-Fc (9a, 9d, and
35 9g); TACI-Fc (9b, 9e, and 9h); or TNFR1-Fc (9c, 9f, and 9i). Cells
were then washed, fixed and the binding of Fc protein was detected by
biotinylated goat anti-human antibody followed by Cy3-streptavidin.
In Figure 9B, COS 7 cells were transfected with TACI (1, 2, and 3);
BCMA (4, 5, and 6); or vector (7, 8, and 9) and incubated with
40 conditioned medium containing AP-TALL-1 (1, 4, and 7), AP-APRIL (2,

5, and 8) or AP-TNF-alpha (3, 6, and 9). Cells were then washed, fixed and stained for AP activity *in situ*. In Figures 9A-9B, TALL-1 is referred to as "BlyS/TALL-1".

5   Figure 10 shows a bar diagram illustrating the results of an IgM ELISA, testing the effects of the indicated cytokines, ligands and receptors on IgM production in target PBLs.

        Figures 11A-11D shows the results of assays demonstrating that
10   interaction between TALL-1 or APRIL and TACI or BCMA results in activation of NF-KB. In Figure 11A, stimulation of TACI/BCMA mediated NF-KB activation by TALL-1 or APRIL. In Figures 11B and 11C, stimulation of TACI/BCMA mediated NF-KB activation by co-transfection of full-length TALL-1 or APRIL. In Figure 11D,
15   treatment of untransfected IM-9 cells with Flag-TALL-1 also resulted in activation of NF-KB, as measured in an electrophoretic mobility shift assay.

        Figure 12 shows a bar diagram illustrating the results of an
20   ELISA testing the effects of blocking TALL-1/TACI and TALL-1/BCMA interactions on NP-specific IgM, low affinity IgG1, and high affinity IgG1 production.

        Figures 13-1 (panels A, B and C) and 13-2 (panels A, B, and C)
25   show immunohistochemical analysis of spleen sections from immunized mice treated with TACI-Fc or BCMA-Fc, and described in further detail in Example 5.

        Figure 14A shows the results of an ELISA testing binding of
30   various anti-APRIL antibodies to Flag-APRIL.

        Figure 14B shows a table indicating the various results of assays of monoclonal antibodies 3C6.4.2; 5E8.7.4; 5E11.1.2; and 5G8.2.2, including isotype analysis.
35
        Figures 15A-15D show bar diagrams illustrating the results of competitive binding ELISAs of anti-APRIL antibodies 3C6.4.2 ("3C6") (Fig. 15A); 5E8.7.4 ("5E8") (Fig. 15B); 5E11.1.2 ("5E11") (Fig. 15C); and 5G8.2.2 ("5G8") (Fig. 15D), and described in further detail in
40   Example 9.

Figures 16A and 16B shows the results of assays demonstrating inhibition of collagen-induced arthritis by TACI-Fc treatment in an *in vivo* murine model. The arthritic score observations are described

5   in Example 10.

Figures 17A-17F show histo-chemical and radiological profiles of joints of CIA mice treated with TACI-Fc or Controls.

10   Figures 18A-18E shows the results of assays demonstrating TACI-Fc inhibited anti-collagen immune responses in the CIA murine model. Figs. 18A and 18B show serum levels of anti-BCII IgG1 and IgG2a isotype antibodies; Fig. 18C illustrates the effects of TACI-Fc on proliferative T cell responses; Fig. 18D illustrates the effects of

15   TACI-Fc on IL-2 production by T cells; Fig. 18E illustrates the effects of TACI-Fc on Interferon-gamma production by T cells.

Figures 19A-19B show the inhibitory effects of TACI-Fc on anti-CD3 induced proliferation of naïve T cells ((Fig. 19A) and on anti-

20   CD3 induced IL-2 production by naïve T cells, as measured in *in vitro* assays.

Figure 20 shows the results of an assay demonstrating inhibition of experimental allergic encephalomyelitis (EAE) by TACI-Fc treatment

25   in an *in vivo* murine model. The clinical EAE score observations are described in Example 11.

DETAILED DESCRIPTION OF THE INVENTION

I.   Definitions

The terms "TACI" or "TACI polypeptide" or "TACI receptor" when

30   used herein encompass "native sequence TACI polypeptides" and "TACI variants" (which are further defined herein). "TACI" is a designation given to those polypeptides which are encoded by the nucleic acid molecules comprising the polynucleotide sequences shown in Figure 1 and variants or fragments thereof, nucleic acid molecules

35   comprising the sequence shown in the Figure 1 and variants thereof as well as fragments of the above. The TACI polypeptides of the invention may be isolated from a variety of sources, such as from human tissue types or from another source, or prepared by recombinant and/or synthetic methods.

A "native sequence" TACI polypeptide comprises a polypeptide
having the same amino acid sequence as the corresponding TACI
polypeptide derived from nature. Such native sequence TACI
polypeptides can be isolated from nature or can be produced by

5   recombinant and/or synthetic means. The term "native sequence TACI
polypeptide" specifically encompasses naturally-occurring truncated
or secreted forms (e.g., an extracellular domain sequence),
naturally-occurring variant forms (e.g., alternatively spliced forms)
and naturally-occurring allelic variants of the polypeptide. The

10 TACI polypeptides of the invention include but are not limited to the
polypeptides described in von Bulow et al., supra and WO98/39361
published September 11, 1998, the spliced variant (referred to as
"hTACI(265)" above and shown in Fig. 6 (SEQ ID NO:9)), and the TACI
polypeptide comprising the contiguous sequence of amino acid residues

15 1-293 of Figs. 1A-1B (SEQ ID NO:2).

A TACI "extracellular domain" or "ECD" refers to a form of the
TACI polypeptide which is essentially free of the transmembrane and
cytoplasmic domains. Ordinarily, a TACI polypeptide ECD will have
less than about 1% of such transmembrane and/or cytoplasmic domains

20 and preferably, will have less than about 0.5% of such domains. It
will be understood that any transmembrane domain(s) identified for
the TACI polypeptides of the present invention are identified
pursuant to criteria routinely employed in the art for identifying
that type of hydrophobic domain. The exact boundaries of a

25 transmembrane domain may vary but most likely by no more than about 5
amino acids at either end of the domain as initially identified. ECD
forms of TACI include those described in von Bulow et al., supra and
WO98/39361.

"TACI variant" means a TACI polypeptide having at least about

30 80% amino acid sequence identity with the amino acid sequence of a
native sequence full length TACI or TACI ECD. Preferably such TACI
variant acts as a TALL-1 antagonist or APRIL antagonist as defined
below. Such TACI variant polypeptides include, for instance, TACI
polypeptides wherein one or more amino acid residues are added, or

35 deleted, at the N- and/or C-terminus, as well as within one or more
internal domains, of the full-length amino acid sequence. Fragments
of the TACI ECD are also contemplated. Ordinarily, a TACI variant
polypeptide will have at least about 80% amino acid sequence
identity, more preferably at least about 81% amino acid sequence

40 identity, more preferably at least about 82% amino acid sequence

13

identity, more preferably at least about 83% amino acid sequence
identity, more preferably at least about 84% amino acid sequence
identity, more preferably at least about 85% amino acid sequence
identity, more preferably at least about 86% amino acid sequence
5   identity, more preferably at least about 87% amino acid sequence
identity, more preferably at least about 88% amino acid sequence
identity, more preferably at least about 89% amino acid sequence
identity, more preferably at least about 90% amino acid sequence
identity, more preferably at least about 91% amino acid sequence
10 identity, more preferably at least about 92% amino acid sequence
identity, more preferably at least about 93% amino acid sequence
identity, more preferably at least about 94% amino acid sequence
identity, more preferably at least about 95% amino acid sequence
identity, more preferably at least about 96% amino acid sequence
15 identity, more preferably at least about 97% amino acid sequence
identity, more preferably at least about 98% amino acid sequence
identity and yet more preferably at least about 99% amino acid
sequence identity with a TACI polypeptide encoded by a nucleic acid
molecule shown in Figure 1 or a specified fragment thereof. TACI
20 variant polypeptides do not encompass the native TACI polypeptide
sequence. Ordinarily, TACI variant polypeptides are at least about
10 amino acids in length, often at least about 20 amino acids in
length, more often at least about 30 amino acids in length, more
often at least about 40 amino acids in length, more often at least
25 about 50 amino acids in length, more often at least about 60 amino
acids in length, more often at least about 70 amino acids in length,
more often at least about 80 amino acids in length, more often at
least about 90 amino acids in length, more often at least about 100
amino acids in length, more often at least about 150 amino acids in
30 length, more often at least about 200 amino acids in length, more
often at least about 250 amino acids in length, more often at least
about 300 amino acids in length, or more.

    The terms "BCMA" or "BCMA polypeptide" or "BCMA receptor" when
used herein encompass "native sequence BCMA polypeptides" and "BCMA
35 variants" (which are further defined herein). "BCMA" is a
designation given to those polypeptides which are encoded by the
nucleic acid molecules comprising the polynucleotide sequences shown
in Figure 2 and variants thereof, nucleic acid molecules comprising
the sequence shown in the Figure 2 and variants thereof as well as
40 fragments of the above. The BCMA polypeptides of the invention may be

14

isolated from a variety of sources, such as from human tissue types
or from another source, or prepared by recombinant and/or synthetic
methods.

A "native sequence" BCMA polypeptide comprises a polypeptide
5   having the same amino acid sequence as the corresponding BCMA
polypeptide derived from nature. Such native sequence BCMA
polypeptides can be isolated from nature or can be produced by
recombinant and/or synthetic means. The term "native sequence BCMA
polypeptide" specifically encompasses naturally-occurring truncated
10   or secreted forms (e.g., an extracellular domain sequence),
naturally-occurring variant forms (e.g., alternatively spliced forms)
and naturally-occurring allelic variants of the polypeptide. The
BCMA polypeptides of the invention include the polypeptides described
in Laabi et al., EMBO J., 11:3897-3904 (1992); Laabi et al., Nucleic
15   Acids Res., 22:1147-1154 (1994); Gras et al., Int. Immunology,
7:1093-1106 (1995); Madry et al., Int. Immunology, 10:1693-1702
(1998); and the BCMA polypeptide comprising the contiguous sequence
of amino acid residues 1-184 of Fig. 2 (SEQ ID NO:4).

A BCMA "extracellular domain" or "ECD" refers to a form of the
20   BCMA polypeptide which is essentially free of the transmembrane and
cytoplasmic domains. Ordinarily, a BCMA polypeptide ECD will have
less than about 1% of such transmembrane and/or cytoplasmic domains
and preferably, will have less than about 0.5% of such domains. It
will be understood that any transmembrane domain(s) identified for
25   the BCMA polypeptides of the present invention are identified
pursuant to criteria routinely employed in the art for identifying
that type of hydrophobic domain. The exact boundaries of a
transmembrane domain may vary but most likely by no more than about 5
amino acids at either end of the domain as initially identified. ECD
30   forms of TACI include those described in Laabi et al., EMBO J.,
11:3897-3904 (1992); Laabi et al., Nucleic Acids Res., 22:1147-1154
(1994); Gras et al., Int. Immunology, 7:1093-1106 (1995); Madry et
al., Int. Immunology, 10:1693-1702 (1998).

"BCMA variant" means a BCMA polypeptide having at least about
35   80% amino acid sequence identity with the amino acid sequence of a
native sequence BCMA or BCMA ECD. Preferably such a BCMA variant
acts as a TALL-1 antagonist or APRIL antagonist as defined below.
Such BCMA variant polypeptides include, for instance, BCMA
polypeptides wherein one or more amino acid residues are added, or
40   deleted, at the N- and/or C-terminus, as well as within one or more

internal domains, of the full-length amino acid sequence. Fragments
of the BCMA ECD are also contemplated. Ordinarily, a BCMA variant
polypeptide will have at least about 80% amino acid sequence
identity, more preferably at least about 81% amino acid sequence

5   identity, more preferably at least about 82% amino acid sequence
identity, more preferably at least about 83% amino acid sequence
identity, more preferably at least about 84% amino acid sequence
identity, more preferably at least about 85% amino acid sequence
identity, more preferably at least about 86% amino acid sequence

10   identity, more preferably at least about 87% amino acid sequence
identity, more preferably at least about 88% amino acid sequence
identity, more preferably at least about 89% amino acid sequence
identity, more preferably at least about 90% amino acid sequence
identity, more preferably at least about 91% amino acid sequence

15   identity, more preferably at least about 92% amino acid sequence
identity, more preferably at least about 93% amino acid sequence
identity, more preferably at least about 94% amino acid sequence
identity, more preferably at least about 95% amino acid sequence
identity, more preferably at least about 96% amino acid sequence

20   identity, more preferably at least about 97% amino acid sequence
identity, more preferably at least about 98% amino acid sequence
identity and yet more preferably at least about 99% amino acid
sequence identity with a BCMA polypeptide encoded by a nucleic acid
molecule shown in Figure 2 or a specified fragment thereof. BCMA

25   variant polypeptides do not encompass the native BCMA polypeptide
sequence. Ordinarily, BCMA variant polypeptides are at least about
10 amino acids in length, often at least about 20 amino acids in
length, more often at least about 30 amino acids in length, more
often at least about 40 amino acids in length, more often at least

30   about 50 amino acids in length, more often at least about 60 amino
acids in length, more often at least about 70 amino acids in length,
more often at least about 80 amino acids in length, more often at
least about 90 amino acids in length, more often at least about 100
amino acids in length, more often at least about 150 amino acids in

35   length, more often at least about 200 amino acids in length, more
often at least about 250 amino acids in length, more often at least
about 300 amino acids in length, or more.

          The terms "TALL-1" or "TALL-1 polypeptide" when used herein
encompass "native sequence TALL-1 polypeptides" and "TALL-1

40   variants". "TALL-1" is a designation given to those polypeptides

                                      16

which are encoded by the nucleic acid molecules comprising the
polynucleotide sequences shown in Figure 3 and variants thereof,
nucleic acid molecules comprising the sequence shown in the Figure 3,
and variants thereof as well as fragments of the above which have the
5   biological activity of the native sequence TALL-1. Variants of TALL-
1 will preferably have at least 80%, more preferably, at least 90%,
and even more preferably, at least 95% amino acid sequence identity
with the native sequence TALL-1 polypeptide shown in Figure 3. A
"native sequence" TALL-1 polypeptide comprises a polypeptide having
10   the same amino acid sequence as the corresponding TALL-1 polypeptide
derived from nature. Such native sequence TALL-1 polypeptides can be
isolated from nature or can be produced by recombinant and/or
synthetic means. The term "native sequence TALL-1 polypeptide"
specifically encompasses naturally-occurring truncated or secreted
15   forms (e.g., an extracellular domain sequence), naturally-occurring
variant forms (e.g., alternatively spliced forms) and naturally-
occurring allelic variants of the polypeptide. The term "TALL-1"
includes those polypeptides described in Shu et al., GenBank
Accession No. AF136293; WO98/18921 published May 7, 1998; EP 869,180
20   published October 7, 1998; WO98/27114 published June 25, 1998;
WO99/12964 published March 18, 1999; WO99/33980 published July 8,
1999; Moore et al., supra; Schneider et al., supra; and Mukhopadhyay
et al., supra.

        The terms "APRIL" or "APRIL polypeptide" when used herein
25   encompass "native sequence APRIL polypeptides" and "APRIL variants".
"APRIL" is a designation given to those polypeptides which are
encoded by the nucleic acid molecules comprising the polynucleotide
sequences shown in Figure 4 and variants thereof, nucleic acid
molecules comprising the sequence shown in the Figure 4, and variants
30   thereof as well as fragments of the above which have the biological
activity of the native sequence APRIL. Variants of APRIL will
preferably have at least 80%, more preferably, at least 90%, and even
more preferably, at least 95% amino acid sequence identity with the
native sequence APRIL polypeptide shown in Figure 4. A "native
35   sequence" APRIL polypeptide comprises a polypeptide having the same
amino acid sequence as the corresponding APRIL polypeptide derived
from nature. Such native sequence APRIL polypeptides can be isolated
from nature or can be produced by recombinant and/or synthetic means.
The term "native sequence APRIL polypeptide" specifically encompasses
40   naturally-occurring truncated or secreted forms (e.g., an

extracellular domain sequence), naturally-occurring variant forms
(e.g., alternatively spliced forms) and naturally-occurring allelic
variants of the polypeptide. The term "APRIL" includes those
polypeptides described in Hahne et al., J. Exp. Med., 188:1185-1190
5   (1998); GenBank Accession No. AF046888; WO 99/00518 published January
7, 1999; WO 99/12965 published March 18, 1999; WO 99/33980 published
July 8, 1999; WO 97/33902 published September 18, 1997; WO 99/11791
published March 11, 1999; EP 911,633 published March 28, 1999; and
WO99/50416 published October 7, 1999.

10   "Percent (%) amino acid sequence identity" with respect to the
ligand or receptor polypeptide sequences identified herein is defined
as the percentage of amino acid residues in a candidate sequence that
are identical with the amino acid residues in such a ligand or
receptor sequence identified herein, after aligning the sequences and
15   introducing gaps, if necessary, to achieve the maximum percent
sequence identity, and not considering any conservative substitutions
as part of the sequence identity. Alignment for purposes of
determining percent amino acid sequence identity can be achieved in
various ways that are within the skill in the art, for instance,
20   using publicly available computer software such as BLAST, BLAST-2,
ALIGN, ALIGN-2 or Megalign (DNASTAR) software. Those skilled in the
art can determine appropriate parameters for measuring alignment,
including any algorithms needed to achieve maximal alignment over the
full-length of the sequences being compared. For purposes herein,
25   however, % amino acid sequence identity values are obtained as
described below by using the sequence comparison computer program
ALIGN-2, wherein the complete source code for the ALIGN-2 program is
provided in the table below. The ALIGN-2 sequence comparison
computer program was authored by Genentech, Inc. and the source code
30   shown in the table below has been filed with user documentation in
the U.S. Copyright Office, Washington D.C., 20559, where it is
registered under U.S. Copyright Registration No. TXU510087. The
ALIGN-2 program is publicly available through Genentech, Inc., South
San Francisco, California or may be compiled from the source code
35   provided in the table below. The ALIGN-2 program should be compiled
for use on a UNIX operating system, preferably digital UNIX V4.0D.
All sequence comparison parameters are set by the ALIGN-2 program and
do not vary.

TABLE - SOURCE CODE

```
      /*
      *
 5    * C-C increased from 12 to 15
      * Z is average of EQ
      * B is average of ND
      * match with stop is _M; stop-stop = 0; J (joker) match = 0
      */
10    #define  _M      -8        /* value of a match with a stop */

      int      _day[26][26] = {
      /*      A  B  C  D  E  F  G  H  I  J  K  L  M  N  O  P  Q  R  S  T  U  V  W  X  Y  Z */
      /* A */ { 2, 0,-2, 0, 0,-4, 1,-1,-1, 0,-1,-2,-1, 0,_M, 1, 0,-2, 1, 1, 0, 0,-6, 0,-3, 0},
15    /* B */ { 0, 3,-4, 3, 2,-5, 0, 1,-2, 0, 0,-3,-2, 2,_M,-1, 1, 0, 0, 0, 0,-2,-5, 0,-3, 1},
      /* C */ {-2,-4,15,-5,-5,-4,-3,-3,-2, 0,-5,-6,-5,-4,_M,-3,-5,-4, 0,-2, 0,-2,-8, 0, 0,-5},
      /* D */ { 0, 3,-5, 4, 3,-6, 1, 1,-2, 0, 0,-4,-3, 2,_M,-1, 2,-1, 0, 0, 0,-2,-7, 0,-4, 2},
      /* E */ { 0, 2,-5, 3, 4,-5, 0, 1,-2, 0, 0,-3,-2, 1,_M,-1, 2,-1, 0, 0, 0,-2,-7, 0,-4, 3},
      /* F */ {-4,-5,-4,-6,-5, 9,-5,-2, 1, 0,-5, 2, 0,-4,_M,-5,-5,-4,-3,-3, 0,-1, 0, 0, 7,-5},
20    /* G */ { 1, 0,-3, 1, 0,-5, 5,-2,-3, 0,-2,-4,-3, 0,_M,-1,-1,-3, 1, 0, 0,-1,-7, 0,-5, 0},
      /* H */ {-1, 1,-3, 1, 1,-2,-2, 6,-2, 0, 0,-2,-2, 2,_M, 0, 3, 2,-1,-1, 0,-2,-3, 0, 0, 2},
      /* I */ {-1,-2,-2,-2, 1,-3,-2, 5, 0,-2, 2, 2,-2,_M,-2,-2,-2,-1, 0, 0, 4,-5, 0,-1,-2},
      /* J */ { 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,_M, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0},
      /* K */ {-1, 0,-5, 0, 0,-5,-2, 0,-2, 0, 5,-3, 0, 1,_M,-1, 1, 3, 0, 0, 0,-2,-3, 0,-4, 0},
25    /* L */ {-2,-3,-6,-4,-3, 2,-4,-2, 2, 0,-3, 6, 4,-3,_M,-3,-2,-3,-3,-1, 0, 2,-2, 0,-1,-2},
      /* M */ {-1,-2,-5,-3,-2, 0,-3,-2, 2, 0, 0, 4, 6,-2,_M,-2,-1, 0,-2,-1, 0, 2,-4, 0,-2,-1},
      /* N */ { 0, 2,-4, 2, 1,-4, 0, 2,-2, 0, 1,-3,-2, 2,_M,-1, 1, 0, 1, 0, 0,-2,-4, 0,-2, 1},
      /* O */ {_M,_M,_M,_M,_M,_M,_M,_M,_M,_M,_M,_M,_M,_M, 0,_M,_M,_M,_M,_M,_M,_M,_M,_M,_M,_M},
      /* P */ { 1,-1,-3,-1,-1,-5,-1, 0,-2, 0,-1,-3,-2,-1,_M, 6, 0, 0, 1, 0, 0,-1,-6, 0,-5, 0},
30    /* Q */ { 0, 1,-5, 2, 2,-5,-1, 3,-2, 0, 1,-2,-1, 1,_M, 0, 4, 1,-1,-1, 0,-2,-5, 0,-4, 3},
      /* R */ {-2, 0,-4,-1,-1,-4,-3, 2,-2, 0, 3,-3, 0, 0,_M, 0, 1, 6, 0,-1, 0,-2, 2, 0,-4, 0},
      /* S */ { 1, 0, 0, 0, 0,-3, 1,-1,-1, 0, 0,-3,-2, 1,_M, 1,-1, 0, 2, 1, 0,-1,-2, 0,-3, 0},
      /* T */ { 1, 0,-2, 0, 0,-3, 0,-1, 0, 0, 0,-1,-1, 0,_M, 0,-1,-1, 1, 3, 0, 0,-5, 0,-3, 0},
      /* U */ { 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,_M, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0},
35    /* V */ { 0,-2,-2,-2,-2,-1,-1,-2, 4, 0,-2, 2, 2,-2,_M,-1,-2,-2,-1, 0, 0, 4,-6, 0,-2,-2},
      /* W */ {-6,-5,-8,-7,-7, 0,-7,-3,-5, 0,-3,-2,-4,-4,_M,-6,-5, 2,-2,-5, 0,-6,17, 0, 0,-6},
      /* X */ { 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,_M, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0},
      /* Y */ {-3,-3, 0,-4,-4, 7,-5, 0,-1, 0,-4,-1,-2,-2,_M,-5,-4,-4,-3,-3, 0,-2, 0, 0,10,-4},
      /* Z */ { 0, 1,-5, 2, 3,-5, 0, 2,-2, 0, 0,-2,-1, 1,_M, 0, 3, 0, 0, 0, 0,-2,-6, 0,-4, 4}
40    };
```

```
/*
*/
#include <stdio.h>
#include <ctype.h>

#define  MAXJMP    16      /* max jumps in a diag */
#define  MAXGAP    24      /* don't continue to penalize gaps larger than this */
#define  JMPS      1024    /* max jmps in an path */
#define  MX        4       /* save if there's at least MX-1 bases since last jmp */

#define  DMAT      3       /* value of matching bases */
#define  DMIS      0       /* penalty for mismatched bases */
#define  DINS0     8       /* penalty for a gap */
#define  DINS1     1       /* penalty per base */
#define  PINS0     8       /* penalty for a gap */
#define  PINS1     4       /* penalty per residue */

struct jmp {
        short            n[MAXJMP];     /* size of jmp (neg for dely) */
        unsigned short   x[MAXJMP];     /* base no. of jmp in seq x */
};                                      /* limits seq to 2^16 -1 */

struct diag {
        int          score;    /* score at last jmp */
        long         offset;   /* offset of prev block */
        short        ijmp;     /* current jmp index */
        struct jmp   jp;       /* list of jmps */
};

struct path {
        int     spc;          /* number of leading spaces */
        short   n[JMPS];/* size of jmp (gap) */
        int     x[JMPS];/* loc of jmp (last elem before gap) */
};

char          *ofile;        /* output file name */
char          *namex[2];     /* seq names: getseqs() */
char          *prog;         /* prog name for err msgs */
char          *seqx[2];      /* seqs: getseqs() */
int           dmax;          /* best diag: nw() */
int           dmax0;         /* final diag */
int           dna;           /* set if dna: main() */
int           endgaps;       /* set if penalizing end gaps */
int           gapx, gapy;    /* total gaps in seqs */
int           len0, len1;    /* seq lens */
int           ngapx, ngapy;  /* total size of gaps */
int           smax;          /* max score: nw() */
int           *xbm;          /* bitmap for matching */
long          offset;        /* current offset in jmp file */
struct diag   *dx;           /* holds diagonals */
struct path   pp[2];         /* holds path for seqs */

char          *calloc(), *malloc(), *index(), *strcpy();
char          *getseq(), *g_calloc();
```

```
/* Needleman-Wunsch alignment program
 *
 * usage: progs file1 file2
 *    where file1 and file2 are two dna or two protein sequences.
 *    The sequences can be in upper- or lower-case an may contain ambiguity
 *    Any lines beginning with ';', '>' or '<' are ignored
 *    Max file length is 65535 (limited by unsigned short x in the jmp struct)
 *    A sequence with 1/3 or more of its elements ACGTU is assumed to be DNA
 *    Output is in the file "align.out"
 *
 * The program may create a tmp file in /tmp to hold info about traceback.
 * Original version developed under BSD 4.3 on a vax 8650
 */
#include "nw.h"
#include "day.h"

static    _dbval[26] = {
        1,14,2,13,0,0,4,11,0,0,12,0,3,15,0,0,0,5,6,8,8,7,9,0,10,0
};

static    _pbval[26] = {
        1, 2|(1<<('D'-'A'))|(1<<('N'-'A')), 4, 8, 16, 32, 64,
        128, 256, 0xFFFFFFF, 1<<10, 1<<11, 1<<12, 1<<13, 1<<14,
        1<<15, 1<<16, 1<<17, 1<<18, 1<<19, 1<<20, 1<<21, 1<<22,
        1<<23, 1<<24, 1<<25|(1<<('E'-'A'))|(1<<('Q'-'A'))
};

main(ac, av)                                                        main
        int     ac;
        char    *av[];
{
        prog = av[0];
        if (ac != 3) {
                fprintf(stderr,"usage: %s file1 file2\n", prog);
                fprintf(stderr,"where file1 and file2 are two dna or two protein sequences.\n");
                fprintf(stderr,"The sequences can be in upper- or lower-case\n");
                fprintf(stderr,"Any lines beginning with ';' or '<' are ignored\n");
                fprintf(stderr,"Output is in the file \"align.out\"\n");
                exit(1);
        }
        namex[0] = av[1];
        namex[1] = av[2];
        seqx[0] = getseq(namex[0], &len0);
        seqx[1] = getseq(namex[1], &len1);
        xbm = (dna)? _dbval : _pbval;

        endgaps = 0;                    /* 1 to penalize endgaps */
        ofile = "align.out";            /* output file */

        nw();           /* fill in the matrix, get the possible jmps */
        readjmps();     /* get the actual jmps */
        print();        /* print stats, alignment */

        cleanup(0);     /* unlink any tmp files */
}
```

```
/* do the alignment, return best score: main()
 * dna: values in Fitch and Smith, PNAS, 80, 1382-1386, 1983
 * pro: PAM 250 values
 * When scores are equal, we prefer mismatches to any gap, prefer
 * a new gap to extending an ongoing gap, and prefer a gap in seqx
 * to a gap in seq y.
 */
nw()                                                                            nw
{
        char        *px, *py;        /* seqs and ptrs */
        int         *ndely, *dely;   /* keep track of dely */
        int         ndelx, delx;     /* keep track of delx */
        int         *tmp;            /* for swapping row0, row1 */
        int         mis;             /* score for each type */
        int         ins0, ins1;      /* insertion penalties */
        register    id;              /* diagonal index */
        register    ij;              /* jmp index */
        register    *col0, *col1;    /* score for curr, last row */
        register    xx, yy;          /* index into seqs */

        dx = (struct diag *)g_calloc("to get diags", len0+len1+1, sizeof(struct diag));

        ndely = (int *)g_calloc("to get ndely", len1+1, sizeof(int));
        dely = (int *)g_calloc("to get dely", len1+1, sizeof(int));
        col0 = (int *)g_calloc("to get col0", len1+1, sizeof(int));
        col1 = (int *)g_calloc("to get col1", len1+1, sizeof(int));
        ins0 = (dna)? DINS0 : PINS0;
        ins1 = (dna)? DINS1 : PINS1;

        smax = -10000;
        if (endgaps) {
                for (col0[0] = dely[0] = -ins0, yy = 1; yy <= len1; yy++) {
                        col0[yy] = dely[yy] = col0[yy-1] - ins1;
                        ndely[yy] = yy;
                }
                col0[0] = 0;          /* Waterman Bull Math Biol 84 */
        }
        else
                for (yy = 1; yy <= len1; yy++)
                        dely[yy] = -ins0;

        /* fill in match matrix
         */
        for (px = seqx[0], xx = 1; xx <= len0; px++, xx++) {
                /* initialize first entry in col
                 */
                if (endgaps) {
                        if (xx == 1)
                                col1[0] = delx = -(ins0+ins1);
                        else
                                col1[0] = delx = col0[0] - ins1;
                        ndelx = xx;
                }
                else {
                        col1[0] = 0;
                        delx = -ins0;
                        ndelx = 0;
                }
```

...nw

```
                    for (py = seqx[1], yy = 1; yy <= len1; py++, yy++) {
                    mis = col0[yy-1];
                    if (dna)
                            mis += (xbm[*px-'A']&xbm[*py-'A'])? DMAT : DMIS;
                    else
                            mis += _day[*px-'A'][*py-'A'];

                    /* update penalty for del in x seq;
                     * favor new del over ongong del
                     * ignore MAXGAP if weighting endgaps
                     */
                    if (endgaps || ndely[yy] < MAXGAP) {
                            if (col0[yy] - ins0 >= dely[yy]) {
                                    dely[yy] = col0[yy] - (ins0+ins1);
                                    ndely[yy] = 1;
                            } else {
                                    dely[yy] -= ins1;
                                    ndely[yy]++;
                            }
                    } else {
                            if (col0[yy] - (ins0+ins1) >= dely[yy]) {
                                    dely[yy] = col0[yy] - (ins0+ins1);
                                    ndely[yy] = 1;
                            } else
                                    ndely[yy]++;
                    }

                    /* update penalty for del in y seq;
                     * favor new del over ongong del
                     */
                    if (endgaps || ndelx < MAXGAP) {
                            if (col1[yy-1] - ins0 >= delx) {
                                    delx = col1[yy-1] - (ins0+ins1);
                                    ndelx = 1;
                            } else {
                                    delx -= ins1;
                                    ndelx++;
                            }
                    } else {
                            if (col1[yy-1] - (ins0+ins1) >= delx) {
                                    delx = col1[yy-1] - (ins0+ins1);
                                    ndelx = 1;
                            } else
                                    ndelx++;
                    }

                    /* pick the maximum score; we're favoring
                     * mis over any del and delx over dely
                     */
```

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```
                            id = xx - yy + len1 - 1;
                            if (mis > = delx && mis > = dely[yy])
 5                                  coll[yy] = mis;
                            else if (delx > = dely[yy]) {
                                    coll[yy] = delx;
                                    ij = dx[id].ijmp;
                                    if (dx[id].jp.n[0] && (!dna || (ndelx > = MAXJMP
10                                  && xx > dx[id].jp.x[ij]+MX) || mis > dx[id].score+DINS0)) {
                                            dx[id].ijmp++;
                                            if (++ij > = MAXJMP) {
                                                    writejmps(id);
                                                    ij = dx[id].ijmp = 0;
15                                                  dx[id].offset = offset;
                                                    offset + = sizeof(struct jmp) + sizeof(offset);
                                            }
                                    }
                                    dx[id].jp.n[ij] = ndelx;
20                                  dx[id].jp.x[ij] = xx;
                                    dx[id].score = delx;
                            }
                            else {
                                    coll[yy] = dely[yy];
25                                  ij = dx[id].ijmp;
                    if (dx[id].jp.n[0] && (!dna || (ndely[yy] > = MAXJMP
                                    && xx > dx[id].jp.x[ij]+MX) || mis > dx[id].score+DINS0)) {
                                            dx[id].ijmp++;
                                            if (++ij > = MAXJMP) {
30                                                  writejmps(id);
                                                    ij = dx[id].ijmp = 0;
                                                    dx[id].offset = offset;
                                                    offset + = sizeof(struct jmp) + sizeof(offset);
                                            }
35                                  }
                                    dx[id].jp.n[ij] = -ndely[yy];
                                    dx[id].jp.x[ij] = xx;
                                    dx[id].score = dely[yy];
                            }
40                          if (xx == len0 && yy < len1) {
                                    /* last col
                                    */
                                    if (endgaps)
                                            coll[yy] -= ins0+ins1*(len1-yy);
45                                  if (coll[yy] > smax) {
                                            smax = coll[yy];
                                            dmax = id;
                                    }
                            }
50                  }
                    if (endgaps && xx < len0)
                            coll[yy-1] -= ins0+ins1*(len0-xx);
                    if (coll[yy-1] > smax) {
                            smax = coll[yy-1];
55                          dmax = id;
                    }
                    tmp = col0; col0 = coll; coll = tmp;
            }
    (void) free((char *)ndely);
60  (void) free((char *)dely);
    (void) free((char *)col0);
    (void) free((char *)coll);                          }
```

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```
/*
 *
 * print() -- only routine visible outside this module
 *
 * static:
 * getmat() -- trace back best path, count matches: print()
 * pr_align() -- print alignment of described in array p[ ]: print()
 * dumpblock() -- dump a block of lines with numbers, stars: pr_align()
 * nums() -- put out a number line: dumpblock()
 * putline() -- put out a line (name, [num], seq, [num]): dumpblock()
 * stars() - -put a line of stars: dumpblock()
 * stripname() -- strip any path and prefix from a seqname
 */

#include "nw.h"

#define SPC        3
#define P_LINE    256        /* maximum output line */
#define P_SPC      3         /* space between name or num and seq */

extern    _day[26][26];
int       olen;              /* set output line length */
FILE      *fx;               /* output file */

print()
{
        int      lx, ly, firstgap, lastgap;        /* overlap */

        if ((fx = fopen(ofile, "w")) == 0) {
                fprintf(stderr, "%s: can't write %s\n", prog, ofile);
                cleanup(1);
        }
        fprintf(fx, "<first sequence: %s (length = %d)\n", namex[0], len0);
        fprintf(fx, "<second sequence: %s (length = %d)\n", namex[1], len1);
        olen = 60;
        lx = len0;
        ly = len1;
        firstgap = lastgap = 0;
        if (dmax < len1 - 1) {          /* leading gap in x */
                pp[0].spc = firstgap = len1 - dmax - 1;
                ly -= pp[0].spc;
        }
        else if (dmax > len1 - 1) {     /* leading gap in y */
                pp[1].spc = firstgap = dmax - (len1 - 1);
                lx -= pp[1].spc;
        }
        if (dmax0 < len0 - 1) {         /* trailing gap in x */
                lastgap = len0 - dmax0 -1;
                lx -= lastgap;
        }
        else if (dmax0 > len0 - 1) {    /* trailing gap in y */
                lastgap = dmax0 - (len0 - 1);
                ly -= lastgap;
        }
        getmat(lx, ly, firstgap, lastgap);
        pr_align();
}
```

print

```
/*
 * trace back the best path, count matches
 */
static
getmat(lx, ly, firstgap, lastgap)
        int       lx, ly;              /* "core" (minus endgaps) */
        int       firstgap, lastgap;   /* leading trailing overlap */
{
        int       nm, i0, i1, siz0, siz1;
        char      outx[32];
        double    pct;
        register  n0, n1;
        register char   *p0, *p1;

        /* get total matches, score
         */
        i0 = i1 = siz0 = siz1 = 0;
        p0 = seqx[0] + pp[1].spc;
        p1 = seqx[1] + pp[0].spc;
        n0 = pp[1].spc + 1;
        n1 = pp[0].spc + 1;

        nm = 0;
        while ( *p0 && *p1 ) {
                if (siz0) {
                        p1++;
                        n1++;
                        siz0--;
                }
                else if (siz1) {
                        p0++;
                        n0++;
                        siz1--;
                }
                else {
                        if (xbm[*p0-'A']&xbm[*p1-'A'])
                                nm++;
                        if (n0++ == pp[0].x[i0])
                                siz0 = pp[0].n[i0++];
                        if (n1++ == pp[1].x[i1])
                                siz1 = pp[1].n[i1++];
                        p0++;
                        p1++;
                }
        }

        /* pct homology:
         * if penalizing endgaps, base is the shorter seq
         * else, knock off overhangs and take shorter core
         */
        if (endgaps)
                lx = (len0 < len1)? len0 : len1;
        else
                lx = (lx < ly)? lx : ly;
        pct = 100.*(double)nm/(double)lx;
        fprintf(fx, "\n");
        fprintf(fx, " < %d match%s in an overlap of %d: %.2f percent similarity\n",
                nm, (nm == 1)? "" : "es", lx, pct);
```

getmat

```
                fprintf(fx, "<gaps in first sequence: %d", gapx);          ...getmat
                if (gapx) {
                        (void) sprintf(outx, " (%d %s%s)",
                                ngapx, (dna)? "base":"residue", (ngapx == 1)? "":"s");
                        fprintf(fx,"%s", outx);

                fprintf(fx, ", gaps in second sequence: %d", gapy);
                if (gapy) {
                        (void) sprintf(outx, " (%d %s%s)",
                                ngapy, (dna)? "base":"residue", (ngapy == 1)? "":"s");
                        fprintf(fx,"%s", outx);
                }
                if (dna)
                        fprintf(fx,
                        "\n<score: %d (match = %d, mismatch = %d, gap penalty = %d + %d per base)\n",
                        smax, DMAT, DMIS, DINS0, DINS1);
                else
                        fprintf(fx,
                        "\n<score: %d (Dayhoff PAM 250 matrix, gap penalty = %d + %d per residue)\n",
                        smax, PINS0, PINS1);
                if (endgaps)
                        fprintf(fx,
                        "<endgaps penalized. left endgap: %d %s%s, right endgap: %d %s%s\n",
                        firstgap, (dna)? "base" : "residue", (firstgap == 1)? "" : "s",
                        lastgap, (dna)? "base" : "residue", (lastgap == 1)? "" : "s");
                else
                        fprintf(fx, "<endgaps not penalized\n");
        }

        static          nm;             /* matches in core -- for checking */
        static          lmax;           /* lengths of stripped file names */
        static          ij[2];          /* jmp index for a path */
        static          nc[2];          /* number at start of current line */
        static          ni[2];          /* current elem number -- for gapping */
        static          siz[2];
        static char     *ps[2];         /* ptr to current element */
        static char     *po[2];         /* ptr to next output char slot */
        static char     out[2][P_LINE]; /* output line */
        static char     star[P_LINE];   /* set by stars() */

        /*
         * print alignment of described in struct path pp[ ]
         */
        static
        pr_align()                                                      pr_align
        {
                int             nn;     /* char count */
                int             more;
                register        i;

                for (i =  0, lmax = 0; i < 2; i++) {
                        nn = stripname(namex[i]);
                        if (nn > lmax)
                                lmax = nn;

                        nc[i] = 1;
                        ni[i] = 1;
                        siz[i] = ij[i] = 0;
                        ps[i] = seqx[i];
                        po[i] = out[i];                 }
```

```
for (nn = nm = 0, more = 1; more; ) {                        ...pr_align
        for (i = more = 0; i < 2; i++) {
                /*
                 * do we have more of this sequence?
                 */
                if (!*ps[i])
                        continue;

                more++;

                if (pp[i].spc) {        /* leading space */
                        *po[i]++ = ' ';
                        pp[i].spc--;
                }
                else if (siz[i]) {      /* in a gap */
                        *po[i]++ = '-';
                        siz[i]--;
                }
                else {                  /* we're putting a seq element
                                         */
                        *po[i] = *ps[i];
                        if (islower(*ps[i]))
                                *ps[i] = toupper(*ps[i]);
                        po[i]++;
                        ps[i]++;

                        /*
                         * are we at next gap for this seq?
                         */
                        if (ni[i] == pp[i].x[ij[i]]) {
                                /*
                                 * we need to merge all gaps
                                 * at this location
                                 */
                                siz[i] = pp[i].n[ij[i]++];
                                while (ni[i] == pp[i].x[ij[i]])
                                        siz[i] += pp[i].n[ij[i]++];
                        }
                        ni[i]++;
                }
        }
        if (++nn == olen || !more && nn) {
                dumpblock();
                for (i = 0; i < 2; i++)
                        po[i] = out[i];
                nn = 0;
        }
}
}

/*
 * dump a block of lines, including numbers, stars: pr_align()
 */
static
dumpblock()                                                  dumpblock
{
        register i;

        for (i = 0; i < 2; i++)
                *po[i]-- = '\0';
```

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...dumpblock

```
                  (void) putc('\n', fx);
 5                for (i = 0; i < 2; i++) {
                          if (*out[i] && (*out[i] != ' ' || *(po[i]) != ' ')) {
                                  if (i == 0)
                                          nums(i);
                                  if (i == 0 && *out[1])
10                                        stars();
                                  putline(i);
                                  if (i == 0 && *out[1])
                                          fprintf(fx, star);
                                  if (i == 1)
15                                        nums(i);
                          }
                  }
          }

20        /*
           * put out a number line: dumpblock()                           '
           */
          static
          nums(ix)                                                        nums
25                int       ix;        /* index in out[ ] holding seq line */
          {
                  char          nline[P_LINE];
                  register      i, j;
                  register char *pn, *px, *py;
30
                  for (pn = nline, i = 0; i < lmax+P_SPC; i++, pn++)
                          *pn = ' ';
                  for (i = nc[ix], py = out[ix]; *py; py++, pn++) {
                          if (*py == ' ' || *py == '-')
35                                *pn = ' ';
                          else {
                                  if (i%10 == 0 || (i == 1 && nc[ix] != 1)) {
                                          j = (i < 0)? -i : i;
                                          for (px = pn; j; j /= 10, px--)
40                                                *px = j%10 + '0';
                                          if (i < 0)
                                                  *px = '-';
                                  }
                                  else
45                                        *pn = ' ';
                                  i++;
                          }
                  }
                  *pn = '\0';
50                nc[ix] = i;
                  for (pn = nline; *pn; pn++)
                          (void) putc(*pn, fx);
                  (void) putc('\n', fx);
          }
55
          /*
           * put out a line (name, [num], seq, [num]): dumpblock()
           */
          static
60        putline(ix)                                                      putline
                  int       ix;                        {
```

...putline

```
        int              i;
        register char    *px;

        for (px = namex[ix], i = 0; *px && *px != ':'; px++, i++)
                (void) putc(*px, fx);
        for (; i < lmax+P_SPC; i++)
                (void) putc(' ', fx);

        /* these count from 1:
         * ni[ ] is current element (from 1)
         * nc[ ] is number at start of current line
         */
        for (px = out[ix]; *px; px++)
                (void) putc(*px&0x7F, fx);
        (void) putc('\n', fx);
}


/*
 * put a line of stars (seqs always in out[0], out[1]): dumpblock()
 */
static
stars()
{
        int                i;
        register char    *p0, *p1, cx, *px;

        if (!*out[0] || (*out[0] == ' ' && *(po[0]) == ' ') ||
           !*out[1] || (*out[1] == ' ' && *(po[1]) == ' '))
                return;
        px = star;
        for (i = lmax+P_SPC; i; i--)
                *px++ = ' ';

        for (p0 = out[0], p1 = out[1]; *p0 && *p1; p0++, p1++) {
                if (isalpha(*p0) && isalpha(*p1)) {
                        if (xbm[*p0-'A']&xbm[*p1-'A']) {
                                cx = '*';
                                nm++;
                        }
                        else if (!dna && _day[*p0-'A'][*p1-'A'] > 0)
                                cx = '.';
                        else
                                cx = ' ';
                }
                else
                        cx = ' ';
                *px++ = cx;
        }
        *px++ = '\n';
        *px = '\0';
}
```

stars

```
/*
 * strip path or prefix from pn, return len: pr_align()
 */
static
stripname(pn)
        char    *pn;        /* file name (may be path) */
{
        register char    *px, *py;

        py = 0;
        for (px = pn; *px; px++)
                if (*px == '/')
                        py = px + 1;
        if (py)
                (void) strcpy(pn, py);
        return(strlen(pn));

}
```

stripname

```
        /*
         * cleanup() -- cleanup any tmp file
         * getseq() -- read in seq, set dna, len, maxlen
 5       * g_calloc() -- calloc() with error checkin
         * readjmps() -- get the good jmps, from tmp file if necessary
         * writejmps() -- write a filled array of jmps to a tmp file: nw()
         */
        #include "nw.h"
10      #include <sys/file.h>

        char    *jname = "/tmp/homgXXXXXX";          /* tmp file for jmps */
        FILE    *fj;

15      int     cleanup();                           /* cleanup tmp file */
        long    lseek();

        /*
         * remove any tmp file if we blow
20       */
        cleanup(i)                                                           cleanup
                int     i;
        {
                if (fj)
25                      (void) unlink(jname);
                exit(i);
        }


        /*
30       * read, return ptr to seq, set dna, len, maxlen
         * skip lines starting with ';', '<', or '>'
         * seq in upper or lower case
         */
        char    *
35      getseq(file, len)                                                    getseq
                char    *file;   /* file name */
                int     *len;    /* seq len */
        {
                char            line[1024], *pseq;
40              register char   *px, *py;
                int             natgc, tlen;
                FILE            *fp;  .

                if ((fp = fopen(file,"r")) == 0) {
45                      fprintf(stderr," %s: can't read %s\n", prog, file);
                        exit(1);
                }
                tlen = natgc = 0;
                while (fgets(line, 1024, fp)) {
50                      if (*line == ';' || *line == '<' || *line == '>')
                                continue;
                        for (px = line; *px != '\n'; px++)
                                if (isupper(*px) || islower(*px))
                                        tlen++;    .
55              }
                if ((pseq = malloc((unsigned)(tlen+6))) == 0) {
                        fprintf(stderr," %s: malloc() failed to get %d bytes for %s\n", prog, tlen+6, file);
                        exit(1);
                }
60              pseq[0] = pseq[1] = pseq[2] = pseq[3] = '\0';
```

```
        py = pseq + 4;
        *len = tlen;
5       rewind(fp);

        while (fgets(line, 1024, fp)) {
                if (*line == ';' || *line == '<' || *line == '>')
                        continue;
10              for (px = line; *px != '\n'; px++) {
                        if (isupper(*px))
                                *py++ = *px;
                        else if (islower(*px))
                                *py++ = toupper(*px);
15                      if (index("ATGCU",*(py-1)))
                                natgc++;
                }
        }
        *py++ = '\0';
20      *py = '\0';
        (void) fclose(fp);
        dna = natgc > (tlen/3);
        return(pseq+4);
}
25
char    *
g_calloc(msg, nx, sz)
        char    *msg;           /* program, calling routine */
        int     nx, sz;         /* number and size of elements */
30 {
        char            *px, *calloc();

        if ((px = calloc((unsigned)nx, (unsigned)sz)) == 0) {
                if (*msg) {
35                      fprintf(stderr, "%s: g_calloc() failed %s (n=%d, sz=%d)\n", prog, msg, nx, sz);
                        exit(1);
                }
        }
        return(px);
40 }


        /*
         * get final jmps from dx[ ] or tmp file, set pp[ ], reset dmax: main()
         */
45 readjmps()
        {
                int             fd = -1;
                int             siz, i0, i1;
                register i, j, xx;
50
                if (fj) {
                        (void) fclose(fj);
                        if ((fd = open(jname, O_RDONLY, 0)) < 0) {
                                fprintf(stderr, "%s: can't open() %s\n", prog, jname);
55                              cleanup(1);
                        }
                }
                for (i = i0 = i1 = 0, dmax0 = dmax, xx = len0; ; i++) {
                        while (1) {
60                              for (j = dx[dmax].ijmp; j >= 0 && dx[dmax].jp.x[j] >= xx; j--)
                                        ;
```

...readjmps

```
                        if (j < 0 && dx[dmax].offset && fj) {
                                (void) lseek(fd, dx[dmax].offset, 0);
                                (void) read(fd, (char *)&dx[dmax].jp, sizeof(struct jmp));
                                (void) read(fd, (char *)&dx[dmax].offset, sizeof(dx[dmax].offset));
                                dx[dmax].ijmp = MAXJMP-1;
                        }
                        else
                                break;
                }
        if (i >= JMPS) {
                fprintf(stderr, "%s: too many gaps in alignment\n", prog);
                cleanup(1);
        }
        if (j >= 0) {
                siz = dx[dmax].jp.n[j];
                xx = dx[dmax].jp.x[j];
                dmax += siz;
                if (siz < 0) {                        /* gap in second seq */
                        pp[1].n[i1] = -siz;
                        xx += siz;
                        /* id = xx - yy + len1 - 1
                        */
                        pp[1].x[i1] = xx - dmax + len1 - 1;
                        gapy++;
                        ngapy -= siz;
/* ignore MAXGAP when doing endgaps */
                        siz = (-siz < MAXGAP || endgaps)? -siz : MAXGAP;
                        i1++;
                }
                else if (siz > 0) {  /* gap in first seq */
                        pp[0].n[i0] = siz;
                        pp[0].x[i0] = xx;
                        gapx++;
                        ngapx += siz;
/* ignore MAXGAP when doing endgaps */
                        siz = (siz < MAXGAP || endgaps)? siz : MAXGAP;
                        i0++;
                }
        }
        else
                break;
}

/* reverse the order of jmps
*/
for (j = 0, i0--; j < i0; j++, i0--) {
        i = pp[0].n[j]; pp[0].n[j] = pp[0].n[i0]; pp[0].n[i0] = i;
        i = pp[0].x[j]; pp[0].x[j] = pp[0].x[i0]; pp[0].x[i0] = i;
}
for (j = 0, i1--; j < i1; j++, i1--) {
        i = pp[1].n[j]; pp[1].n[j] = pp[1].n[i1]; pp[1].n[i1] = i;
        i = pp[1].x[j]; pp[1].x[j] = pp[1].x[i1]; pp[1].x[i1] = i;
}
if (fd >= 0)
        (void) close(fd);
if (fj) {
        (void) unlink(jname);
        fj = 0;
        offset = 0;
}                                       }
```

```
        /*
         * write a filled jmp struct offset of the prev one (if any): nw()
5       */
        writejmps(ix)                                                              writejmps
                int     ix;
        {
                char    *mktemp();
10
                if (!fj) {
                        if (mktemp(jname) < 0) {
                                fprintf(stderr, "%s: can't mktemp() %s\n", prog, jname);
                                cleanup(1);
15                      }
                        if ((fj = fopen(jname, "w")) == 0) {
                                fprintf(stderr, "%s: can't write %s\n", prog, jname);
                                exit(1);
                        }
20              }
                (void) fwrite((char *)&dx[ix].jp, sizeof(struct jmp), 1, fj);
                (void) fwrite((char *)&dx[ix].offset, sizeof(dx[ix].offset), 1, fj);
        }

25
```

For purposes herein, the % amino acid sequence identity of a
given amino acid sequence A to, with, or against a given amino acid
sequence B (which can alternatively be phrased as a given amino acid
sequence A that has or comprises a certain % amino acid sequence
5   identity to, with, or against a given amino acid sequence B) is
calculated as follows:

                      100 times the fraction X/Y

10   where X is the number of amino acid residues scored as identical
matches by the sequence alignment program ALIGN-2 in that program's
alignment of A and B, and where Y is the total number of amino acid
residues in B. It will be appreciated that where the length of amino
acid sequence A is not equal to the length of amino acid sequence B,
15   the % amino acid sequence identity of A to B will not equal the %
amino acid sequence identity of B to A. As examples of % amino acid
sequence identity calculations, Figures 5A-5B demonstrate how to
calculate the % amino acid sequence identity of the amino acid
sequence designated "Comparison Protein" to the amino acid sequence
20   designated "PRO".

        Unless specifically stated otherwise, all % amino acid sequence
identity values used herein are obtained as described above using the
ALIGN-2 sequence comparison computer program. However, % amino acid
sequence identity may also be determined using the sequence
25   comparison program NCBI-BLAST2 (Altschul et al., Nucleic Acids Res.
25:3389-3402 (1997)). The NCBI-BLAST2 sequence comparison program
may be downloaded from the NCBI internet web site. NCBI-BLAST2 uses
several search parameters, wherein all of those search parameters are
set to default values including, for example, unmask = yes, strand =
30   all, expected occurrences = 10, minimum low complexity length = 15/5,
multi-pass e-value = 0.01, constant for multi-pass = 25, dropoff for
final gapped alignment = 25 and scoring matrix = BLOSUM62.

        In situations where NCBI-BLAST2 is employed for amino acid
sequence comparisons, the % amino acid sequence identity of a given
35   amino acid sequence A to, with, or against a given amino acid
sequence B (which can alternatively be phrased as a given amino acid
sequence A that has or comprises a certain % amino acid sequence
identity to, with, or against a given amino acid sequence B) is
calculated as follows:

40

                                  36

100 times the fraction X/Y

where X is the number of amino acid residues scored as identical
matches by the sequence alignment program NCBI-BLAST2 in that
5   program's alignment of A and B, and where Y is the total number of
amino acid residues in B. It will be appreciated that where the
length of amino acid sequence A is not equal to the length of amino
acid sequence B, the % amino acid sequence identity of A to B will
not equal the % amino acid sequence identity of B to A.

10   The term "epitope tagged" when used herein refers to a chimeric
polypeptide comprising a polypeptide fused to a "tag polypeptide".
The tag polypeptide has enough residues to provide an epitope against
which an antibody can be made. The tag polypeptide preferably also
is fairly unique so that the antibody does not substantially cross-
15   react with other epitopes. Suitable tag polypeptides generally have
at least six amino acid residues and usually between about 8 and 50
amino acid residues (preferably, between about 10 and 20 amino acid
residues).

      As used herein, the term "immunoadhesin" designates antibody-
20   like molecules which combine the binding specificity of a
heterologous protein (an "adhesin") with the effector functions of
immunoglobulin constant domains. Structurally, the immunoadhesins
comprise a fusion of an amino acid sequence with the desired binding
specificity which is other than the antigen recognition and binding
25   site of an antibody (i.e., is "heterologous"), and an immunoglobulin
constant domain sequence. The adhesin part of an immunoadhesin
molecule typically is a contiguous amino acid sequence comprising at
least the binding site of a receptor or a ligand. The immunoglobulin
constant domain sequence in the immunoadhesin may be obtained from
30   any immunoglobulin, such as IgG-1, IgG-2, IgG-3, or IgG-4 subtypes,
IgA (including IgA-1 and IgA-2), IgE, IgD or IgM.

      The term "antagonist" is used in the broadest sense, and
includes any molecule that partially or fully blocks, inhibits, or
neutralizes one or more biological activities of TALL-1 polypeptide,
35   APRIL polypeptide, or both TALL-1 and APRIL, in vitro, in situ, or in
vivo. Examples of such biological activities of TALL-1 and APRIL
polypeptides include binding of TALL-1 or APRIL to TACI or BCMA,
activation of NF-KB and activation of proliferation and of Ig
secretion by B cells, immune-related conditions such as rheumatoid
40   arthritis, as well as those further reported in the literature. An

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antagonist may function in a direct or indirect manner. For
instance, the antagonist may function to partially or fully block,
inhibit or neutralize one or more biological activities of TALL-1
polypeptide, APRIL polypeptide, or both TALL-1 and APRIL, *in vitro*,
5   *in situ*, or *in vivo* as a result of its direct binding to TALL-1,
APRIL, TACI or BCMA. The antagonist may also function indirectly to
partially or fully block, inhibit or neutralize one or more
biological activities of TALL-1 polypeptide, APRIL polypeptide, or
both TALL-1 and APRIL, *in vitro*, *in situ*, or *in vivo* as a result of,
10 e.g., blocking or inhibiting another effector molecule. The
antagonist molecule may comprise a "dual" antagonist activity wherein
the molecule is capable of partially or fully blocking, inhibiting or
neutralizing a biological activity of both TALL-1 and APRIL.

The term "agonist" is used in the broadest sense, and includes
15 any molecule that partially or fully enhances, stimulates or
activates one or more biological activities of TACI polypeptide, BCMA
polypeptide, or both TACI and BCMA, *in vitro*, *in situ*, or *in vivo*.
Examples of such biological activities of TACI and BCMA include
activation of NF-KB, induction of immunoglobulin production and
20 secretion, and cell proliferation, as well as those further reported
in the literature. An agonist may function in a direct or indirect
manner. For instance, the agonist may function to partially or fully
enhance, stimulate or activate one or more biological activities of
TACI polypeptide, BCMA polypeptide, or both TACI and BCMA, *in vitro*,
25 *in situ*, or *in vivo* as a result of its direct binding to TACI or
BCMA, which causes receptor activation or signal transduction. The
agonist may also function indirectly to partially or fully enhance,
stimulate or activate one or more biological activities of TACI
polypeptide, BCMA polypeptide, or both TACI and BCMA, *in vitro*, *in*
30 *situ*, or *in vivo* as a result of, e.g., stimulating another effector
molecule which then causes TACI or BCMA receptor activation or signal
transduction. It is contemplated that an agonist may act as an
enhancer molecule which functions indirectly to enhance or increase
TACI or BCMA activation or activity. For instance, the agonist may
35 enhance activity of endogenous TALL-1 or APRIL in a mammal. This
could be accomplished, for example, by pre-complexing TACI or BCMA or
by stabilizing complexes of the respective ligand with the TACI or
BCMA receptor (such as stabilizing native complex formed between
TALL-1 and TACI or APRIL and TACI).

The term "TALL-1 antagonist" or "APRIL antagonist" refers to any molecule that partially or fully blocks, inhibits, or neutralizes a biological activity of TALL-1 or APRIL, respectively, or both TALL-1 and APRIL, and include, but are not limited to, soluble forms of TACI

5   receptor or BCMA receptor such as an extracellular domain sequence of TACI or BCMA, TACI receptor immunoadhesins, BCMA receptor immunoadhesins, TACI receptor fusion proteins, BCMA receptor fusion proteins, covalently modified forms of TACI receptor, covalently modified forms of BCMA receptor, TACI variants, BCMA variants, TACI

10 receptor antibodies, BCMA receptor antibodies, TALL-1 antibodies, and APRIL antibodies. To determine whether a TALL-1 antagonist molecule partially or fully blocks, inhibits or neutralizes a biological activity of TALL-1 or APRIL, assays may be conducted to assess the effect(s) of the antagonist molecule on, for example, binding of TALL-1

15 or APRIL to TACI or to BCMA, or NF-KB activation by the respective ligand. Such assays may be conducted in known *in vitro* or *in vivo* assay formats, for instance, in cells expressing BCMA and/or TACI. Preferably, the TALL-1 antagonist employed in the methods described herein will be capable of blocking or neutralizing at least one type of

20 TALL-1 activity, which may optionally be determined in assays such as described herein. To determine whether an APRIL antagonist molecule partially or fully blocks, inhibits or neutralizes a biological activity of TALL-1 or APRIL, assays may be conducted to assess the effect(s) of the antagonist molecule on, for example, binding of TALL-1

25 or APRIL to TACI or to BCMA, or NF-KB activation by the ligand. Such assays may be conducted in known *in vitro* or *in vivo* formats, for instance, using cells transfected with TACI or BCMA (or both TACI and BCMA) (preferably transfected at relatively low levels). Preferably, the APRIL antagonist employed in the methods described herein will be

30 capable of blocking or neutralizing at least one type of APRIL activity, which may optionally be determined in a binding assay or an IgM-production assay. Optionally, a TALL-1 antagonist or APRIL antagonist will be capable of reducing or inhibiting binding of either TALL-1 or APRIL (or both TALL-1 and APRIL) to TACI or to BCMA by at

35 least 50%, preferably, by at least 90%, more preferably by at least 99%, and most preferably, by 100%, as compared to a negative control molecule, in a binding assay. In one embodiment, the TALL-1 antagonist or APRIL antagonist will comprise antibodies which will competitively inhibit the binding of another ligand or antibody to TACI or BCMA.

40 Methods for determining antibody specificity and affinity by

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competitive inhibition are known in the art [see, e.g., Harlow et al.,
Antibodies:A Laboratory Manual, Cold Spring Harbor Laboratory Press,
Cold Spring Harbor, NY (1998); Colligan et al., _Current Protocols in_
Immunology, Green Publishing Assoc., NY (1992; 1993); Muller, _Meth._
5   _Enzym._, _92_:589-601 (1983).

The term "TACI agonist" or "BCMA agonist" refers to any molecule
that partially or fully enhances, stimulates or activates a biological
activity of TACI or BCMA, respectively, or both TACI and BCMA, and
include, but are not limited to, anti-TACI receptor antibodies and
10   anti-BCMA receptor antibodies. To determine whether a TACI agonist
molecule partially or fully enhances, stimulates, or activates a
biological activity of TACI or BCMA, assays may be conducted to assess
the effect(s) of the agonist molecule on, for example, PBLs or TACI or
BCMA-transfected cells. Such assays may be conducted in known _in vitro_
15   or _in vivo_ assay formats. Preferably, the TACI agonist employed in the
methods described herein will be capable of enhancing or activating at
least one type of TACI activity, which may optionally be determined in
assays such as described herein. To determine whether a BCMA agonist
molecule partially or fully enhances, stimulates, or activates a
20   biological activity of TACI or BCMA, assays may be conducted to assess
the effect(s) of the antagonist molecule on, for example, an activity
of APRIL or TACI. Such assays may be conducted in _in vitro_ or _in vivo_
formats, for instance, using PBLs or TACI or BCMA-transfected cells.
Preferably, the TACI agonist or BCMA agonist will be capable of
25   stimulating or activating TACI or BCMA, respectively, to the extent of
that accomplished by the native ligand for the TACI or BCMA receptors.

The term "antibody" is used in the broadest sense and
specifically covers, for example, single monoclonal antibodies
against TALL-1, APRIL, TACI, or BCMA, antibody compositions with
30   polyepitopic specificity, single chain antibodies, and fragments of
antibodies.

The term "monoclonal antibody" as used herein refers to an
antibody obtained from a population of substantially homogeneous
antibodies, _i.e._, the individual antibodies comprising the population
35   are identical except for possible naturally occurring mutations that
may be present in minor amounts. Monoclonal antibodies are highly
specific, being directed against a single antigenic site.
Furthermore, in contrast to conventional (polyclonal) antibody
preparations which typically include different antibodies directed
40   against different determinants (epitopes), each monoclonal antibody

is directed against a single determinant on the antigen. In addition
to their specificity, the monoclonal antibodies are advantageous in
that they are synthesized by the hybridoma culture, uncontaminated by
other immunoglobulins. The modifier "monoclonal" indicates the

5   character of the antibody as being obtained from a substantially
homogeneous population of antibodies, and is not to be construed as
requiring production of the antibody by any particular method. For
example, the monoclonal antibodies to be used in ,accordance with the
present invention may be made by the hybridoma method first described

10   by Kohler et al., Nature, 256:495 (1975), or may be made by
recombinant DNA methods (see, e.g., U.S. Patent No. 4,816,567). The
"monoclonal antibodies" may also be isolated from phage antibody
libraries using the techniques described in Clackson et al., Nature,
352:624-628 (1991) and Marks et al., J. Mol. Biol., 222:581-597

15   (1991), for example.

The monoclonal antibodies herein specifically include "chimeric"
antibodies (immunoglobulins) in which a portion of the heavy and/or
light chain is identical with or homologous to corresponding
sequences in antibodies derived from a particular species or

20   belonging to a particular antibody class or subclass, while the
remainder of the chain(s) is identical with or homologous to
corresponding sequences in antibodies derived from another species or
belonging to another antibody class or subclass, as well as fragments
of such antibodies, so long as they exhibit the desired biological

25   activity (U.S. Patent No. 4,816,567; Morrison et al., Proc. Natl.
Acad. Sci. USA, 81:6851-6855 (1984)). Methods of making chimeric
antibodies are known in the art.

"Humanized" forms of non-human (e.g., murine) antibodies are
chimeric immunoglobulins, immunoglobulin chains or fragments thereof

30   (such as Fv, Fab, Fab', F(ab')$_2$ or other antigen-binding subsequences
of antibodies) which contain minimal sequence derived from non-human
immunoglobulin. For the most part, humanized antibodies are human
immunoglobulins (recipient antibody) in which residues from a
complementarity-determining region (CDR) of the recipient are

35   replaced by residues from a CDR of a non-human species (donor
antibody) such as mouse, rat or rabbit having the desired
specificity, affinity, and capacity. In some instances, Fv framework
region (FR) residues of the human immunoglobulin are replaced by
corresponding non-human residues. Furthermore, humanized antibodies

40   may comprise residues which are found neither in the recipient

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antibody nor in the imported CDR or framework sequences. These modifications are made to further refine and maximize antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones et al., Nature, 321:522-525 (1986); Reichmann et al., Nature, 332:323-329 (1988); and Presta, Curr. Op. Struct. Biol., 2:593-596 (1992). The humanized antibody includes a PRIMATIZED™ antibody wherein the antigen-binding region of the antibody is derived from an antibody produced by immunizing macaque monkeys with the antigen of interest. Methods of making humanized antibodies are known in the art.

Human antibodies can also be produced using various techniques known in the art, including phage-display libraries. Hoogenboom and Winter, J. Mol. Biol., 227:381 (1991); Marks et al., J. Mol. Biol., 222:581 (1991). The techniques of Cole et al. and Boerner et al. are also available for the preparation of human monoclonal antibodies. Cole et al., Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, p. 77 (1985); Boerner et al., J. Immunol., 147(1):86-95 (1991).

"Isolated," when used to describe the various proteins disclosed herein, means protein that has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials that would typically interfere with diagnostic or therapeutic uses for the protein, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In preferred embodiments, the protein will be purified (1) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (2) to homogeneity by SDS-PAGE under non-reducing or reducing conditions using Coomassie blue or, preferably, silver stain. Isolated protein includes protein in situ within recombinant cells, since at least one component of the protein natural environment will not be present. Ordinarily, however, isolated protein will be prepared by at least one purification step.

"Treatment" or "therapy" refer to both therapeutic treatment and prophylactic or preventative measures.

"Mammal" for purposes of treatment or therapy refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cats, cows, etc. Preferably, the mammal is human.

"TALL-1 -related pathological condition" and "APRIL-related pathological condition" refer to pathologies or conditions associated with abnormal levels of expression or activity of TALL-1 or APRIL, respectively, in excess of, or less than, levels of expression or activity in normal healthy mammals, where such excess or diminished levels occur in a systemic, localized, or particular tissue or cell type or location in the body. TALL-1 -related pathological conditions and APRIL-related pathological conditions include acute and chronic immune related diseases and cancer.

The terms "cancer", "cancerous", and "malignant" refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include but are not limited to, carcinoma including adenocarcinoma, lymphoma, blastoma, melanoma, sarcoma, and leukemia. More particular examples of such cancers include squamous cell cancer, small-cell lung cancer, non-small cell lung cancer, gastrointestinal cancer, Hodgkin's and non-Hodgkin's lymphoma, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer such as hepatic carcinoma and hepatoma, bladder cancer, breast cancer, colon cancer, colorectal cancer, endometrial carcinoma, salivary gland carcinoma, kidney cancer such as renal cell carcinoma and Wilms' tumors, basal cell carcinoma, melanoma, prostate cancer, vulval cancer, thyroid cancer, testicular cancer, esophageal cancer, and various types of head and neck cancer. Optionally, the cancer will express, or have associated with the cancer cell, TALL-1, APRIL, TACI or BCMA. By way of example, colon, lung and melanoma cancers have been reported in the literature to express APRIL. The preferred cancers for treatment herein include lymphoma, leukemia and myeloma, and subtypes thereof, such as Burkitt's lymphoma, multiple myeloma, acute lymphoblastic or lymphocytic leukemia, non-Hodgkin's and Hodgkin's lymphoma, and acute myeloid leukemia.

The term "immune related disease" means a disease in which a component of the immune system of a mammal causes, mediates or otherwise contributes to a morbidity in the mammal. Also included

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are diseases in which stimulation or intervention of the immune
response has an ameliorative effect on progression of the disease.
Included within this term are autoimmune diseases, immune-mediated
inflammatory diseases, non-immune-mediated inflammatory diseases,

5   infectious diseases, and immunodeficiency diseases. Examples of
immune-related and inflammatory diseases, some of which are immune or
T cell mediated, which can be treated according to the invention
include systemic lupus erythematosis, rheumatoid arthritis, juvenile
chronic arthritis, spondyloarthropathies, systemic sclerosis

10   (scleroderma), idiopathic inflammatory myopathies (dermatomyositis,
polymyositis), Sjogren's syndrome, systemic vasculitis, sarcoidosis,
autoimmune hemolytic anemia (immune pancytopenia, paroxysmal
nocturnal hemoglobinuria), autoimmune thrombocytopenia (idiopathic
thrombocytopenic purpura, immune-mediated thrombocytopenia),

15   thyroiditis (Grave's disease, Hashimoto's thyroiditis, juvenile
lymphocytic thyroiditis, atrophic thyroiditis), diabetes mellitus,
immune-mediated renal disease (glomerulonephritis, tubulointerstitial
nephritis), demyelinating diseases of the central and peripheral
nervous systems such as multiple sclerosis, idiopathic demyelinating

20   polyneuropathy or Guillain-Barré syndrome, and chronic inflammatory
demyelinating polyneuropathy, hepatobiliary diseases such as
infectious hepatitis (hepatitis A, B, C, D, E and other non-
hepatotropic viruses), autoimmune chronic active hepatitis, primary
biliary cirrhosis, granulomatous hepatitis, and sclerosing

25   cholangitis, inflammatory and fibrotic lung diseases such as
inflammatory bowel disease (ulcerative colitis: Crohn's disease),
gluten-sensitive enteropathy, and Whipple's disease, autoimmune or
immune-mediated skin diseases including bullous skin diseases,
erythema multiforme and contact dermatitis, psoriasis, allergic

30   diseases such as asthma, allergic rhinitis, atopic dermatitis, food
hypersensitivity and urticaria, immunologic diseases of the lung such
as eosinophilic pneumonias, idiopathic pulmonary fibrosis and
hypersensitivity pneumonitis, transplantation associated diseases
including graft rejection and graft-versus-host-disease. Infectious

35   diseases include AIDS (HIV infection), hepatitis A, B, C, D, and E,
bacterial infections, fungal infections, protozoal infections and
parasitic infections.

        "Autoimmune disease" is used herein in a broad, general sense to
refer to disorders or conditions in mammals in which destruction of
40   normal or healthy tissue arises from humoral or cellular immune

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responses of the individual mammal to his or her own tissue constituents.   Examples include, but are not limited to, lupus erythematous, thyroiditis, rheumatoid arthritis, psoriasis, multiple sclerosis, autoimmune diabetes, and inflammatory bowel disease (IBD).

The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents the function of cells and/or causes destruction of cells.   The term is intended to include radioactive isotopes (e.g. I^{131}, I^{125}, Y^{90} and Re^{186}), chemotherapeutic agents, and toxins such as enzymatically active toxins of bacterial, fungal, plant or animal origin, or fragments thereof.

A "chemotherapeutic agent" is a chemical compound useful in the treatment of disease.   Examples of chemotherapeutic agents include adriamycin, doxorubicin, epirubicin, 5-fluorouracil, cytosine arabinoside ("Ara-C"), cyclophosphamide, thiotepa, busulfan, cytoxin, taxoids, e.g. paclitaxel (Taxol, Bristol-Myers Squibb Oncology, Princeton, NJ), and doxetaxel (Taxotere, Rhône-Poulenc Rorer, Antony, Rnace), toxotere, methotrexate, cisplatin, melphalan, CPT-11, vinblastine, bleomycin, etoposide, ifosfamide, mitomycin C, mitoxantrone, vincristine, vinorelbine, carboplatin, teniposide, daunomycin, carminomycin, aminopterin, dactinomycin, mitomycins, esperamicins (see U.S. Pat. No. 4,675,187), melphalan and other related nitrogen mustards.   Also included in this definition are hormonal agents that act to regulate or inhibit hormone action such as tamoxifen and onapristone.

A "growth inhibitory agent" when used herein refers to a compound or composition which inhibits growth of a cell, either *in vitro* or *in vivo*.   Thus, the growth inhibitory agent is one which significantly reduces the percentage of cells overexpressing such genes in S phase.   Examples of growth inhibitory agents include agents that block cell cycle progression (at a place other than S phase), such as agents that induce G1 arrest and M-phase arrest. Classical M-phase blockers include the vincas (vincristine and vinblastine), taxol, and topo II inhibitors such as doxorubicin, epirubicin, daunorubicin, etoposide, and bleomycin.   Those agents that arrest G1 also spill over into S-phase arrest, for example, DNA alkylating agents such as tamoxifen, prednisone, dacarbazine, mechlorethamine, cisplatin, methotrexate, 5-fluorouracil, and ara-C. Further information can be found in *The Molecular Basis of Cancer*, Mendelsohn and Israel, eds., Chapter 1, entitled "Cell cycle

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regulation, oncogens, and antineoplastic drugs" by Murakami *et al.*
(WB Saunders: Philadelphia, 1995), especially p. 13.

The term "cytokine" is a generic term for proteins released by
one cell population which act on another cell as intercellular

5   mediators. Examples of such cytokines are lymphokines, monokines,
and traditional polypeptide hormones. Included among the cytokines
are growth hormone such as human growth hormone, N-methionyl human
growth hormone, and bovine growth hormone; parathyroid hormone;
thyroxine; insulin; proinsulin; relaxin; prorelaxin; glycoprotein

10   hormones such as follicle stimulating hormone (FSH), thyroid
stimulating hormone (TSH), and luteinizing hormone (LH); hepatic
growth factor; fibroblast growth factor; prolactin; placental
lactogen; tumor necrosis factor-α and -β; mullerian-inhibiting
substance; mouse gonadotropin-associated peptide; inhibin; activin;

15   vascular endothelial growth factor; integrin; thrombopoietin (TPO);
nerve growth factors; platelet-growth factor; transforming growth
factors (TGFs) such as TGF-α and TGF-β; insulin-like growth factor-I
and -II; erythropoietin (EPO); osteoinductive factors; interferons
such as interferon-α, -β, and -gamma; colony stimulating factors

20   (CSFs) such as macrophage-CSF (M-CSF); granulocyte-macrophage-CSF
(GM-CSF); and granulocyte-CSF (G-CSF); interleukins (ILs) such as IL-
1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-11, IL-12; and
other polypeptide factors including LIF and kit ligand (KL). As used
herein, the term cytokine includes proteins from natural sources or

25   from recombinant cell culture and biologically active equivalents of
the native sequence cytokines.

II.   Methods and Materials

Generally, the methods of the invention for modulating TALL-1,
30   APRIL, TACI, and/or BCMA activity in mammalian cells comprise exposing
the cells to a desired amount of antagonist or agonist which affects
TALL-1 or APRIL interaction with TACI or BCMA. Preferably, the amount
of antagonist or agonist employed will be an amount effective to affect
the binding and/or activity of the respective ligand or respective
35   receptor to achieve a therapeutic effect. This can be accomplished *in
vivo* or *ex vivo* in accordance, for instance, with the methods described
below and in the Examples. Exemplary conditions or disorders to be
treated with such TALL-1 antagonists or APRIL antagonists include
conditions in mammals clinically referred to as autoimmune diseases,

including but not limited to rheumatoid arthritis, multiple
sclerosis, psoriasis, and lupus or other pathological conditions in
which B cell response(s) in mammals is abnormally upregulated such as
cancer. As shown in the Examples below, TACI immunoadhesin molecules
5   substantially inhibited arthritic disease induced by immunization
with type-II collagen, reduced joint inflammation and formation of
anti-collagen antibodies, prevented bone and cartilage destruction
and blocked stimulation of autoreactive T cells. These results
implicate TACI in T cell-mediated autoimmunity, and suggest that
10 blocking or inhibiting TACI interactions with TALL-1 and/or APRIL may
have therapeutic utility for autoimmune diseases such as RA.
Exemplary conditions or disorders to be treated with TACI agonists or
BCMA agonists include immunodeficiency and cancer.

        Diagnostic methods are also provided herein. For instance, the
15 antagonists or agonists may be employed to detect the respective
ligands (TALL-1 or APRIL) or receptors (TACI or BCMA) in mammals
known to be or suspected of having a TALL-1 - related pathological
condition or APRIL-related pathological condition. The antagonist or
agonist molecule may be used, e.g., in immunoassays to detect or
20 quantitate TALL-1 or APRIL in a sample. A sample, such as cells
obtained from a mammal, can be incubated in the presence of a labeled
antagonist or agonist molecule, and detection of the labeled
antagonist or agonist bound in the sample can be performed. Such
assays, including various clinical assay procedures, are known in the
25 art, for instance as described in Voller et al., Immunoassays,
University Park, 1981.

        A.   MATERIALS
        The antagonists and agonists which can be employed in the
30 methods include, but are not limited to, soluble forms of TACI and
BCMA receptors, TACI receptor immunoadhesins and BCMA receptor
immunoadhesins, fusion-proteins comprising TACI or BCMA, covalently
modified forms of TACI or BCMA, TACI receptor variants and BCMA
receptor variants, TACI or BCMA receptor antibodies, and TALL-1 or
35 APRIL antibodies. Various techniques that can be employed for making
the antagonists and agonists are described below.

        Generally, the compositions of the invention may be prepared
using recombinant techniques known in the art. The description below
relates to methods of producing such polypeptides by culturing host
40 cells transformed or transfected with a vector containing the

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encoding nucleic acid and recovering the polypeptide from the cell
culture. (See, e.g., Sambrook et al., <u>Molecular Cloning: A Laboratory
Manual</u> (New York: Cold Spring Harbor Laboratory Press, 1989);
Dieffenbach et al., <u>PCR Primer:A Laboratory Manual</u> (Cold Spring
5   Harbor Laboratory Press, 1995)).

The nucleic acid (e.g., cDNA or genomic DNA) encoding the
desired polypeptide may be inserted into a replicable vector for
further cloning (amplification of the DNA) or for expression.
Various vectors are publicly available. The vector components
10   generally include, but are not limited to, one or more of the
following: a signal sequence, an origin of replication, one or more
marker genes, an enhancer element, a promoter, and a transcription
termination sequence, each of which is described below. Optional
signal sequences, origins of replication, marker genes, enhancer
15   elements and transcription terminator sequences that may be employed
are known in the art and described in further detail in WO97/25428.

Expression and cloning vectors usually contain a promoter that
is recognized by the host organism and is operably linked to the
encoding nucleic acid sequence. Promoters are untranslated sequences
20   located upstream (5') to the start codon of a structural gene
(generally within about 100 to 1000 bp) that control the
transcription and translation of a particular nucleic acid sequence,
to which they are operably linked. Such promoters typically fall
into two classes, inducible and constitutive. Inducible promoters
25   are promoters that initiate increased levels of transcription from
DNA under their control in response to some change in culture
conditions, e.g., the presence or absence of a nutrient or a change
in temperature. At this time a large number of promoters recognized
by a variety of potential host cells are well known. These promoters
30   are operably linked to the encoding DNA by removing the promoter from
the source DNA by restriction enzyme digestion and inserting the
isolated promoter sequence into the vector.

Promoters suitable for use with prokaryotic and eukaryotic hosts
are known in the art, and are described in further detail in
35   WO97/25428.

Construction of suitable vectors containing one or more of the
above-listed components employs standard ligation techniques.
Isolated plasmids or DNA fragments are cleaved, tailored, and re-
ligated in the form desired to generate the plasmids required. For
40   analysis to confirm correct sequences in plasmids constructed, the

48

ligation mixtures can be used to transform *E. coli* K12 strain 294
(ATCC 31,446) and successful transformants selected by ampicillin or
tetracycline resistance where appropriate. Plasmids from the
transformants are prepared, analyzed by restriction endonuclease
5   digestion, and/or sequenced using standard techniques known in the
art. [See, e.g., Messing et al., Nucleic Acids Res., 9:309 (1981);
Maxam et al., Methods in Enzymology, 65:499 (1980)].

Expression vectors that provide for the transient expression in
mammalian cells of the encoding DNA may be employed. In general,
10 transient expression involves the use of an expression vector that is
able to replicate efficiently in a host cell, such that the host cell
accumulates many copies of the expression vector and, in turn,
synthesizes high levels of a desired polypeptide encoded by the
expression vector [Sambrook et al., supra]. Transient expression
15 systems, comprising a suitable expression vector and a host cell,
allow for the convenient positive identification of polypeptides
encoded by cloned DNAs, as well as for the rapid screening of such
polypeptides for desired biological or physiological properties.

Other methods, vectors, and host cells suitable for adaptation
20 to the synthesis of the desired polypeptide in recombinant vertebrate
cell culture are described in Gething et al., Nature, 293:620-625
(1981); Mantei et al., Nature, 281:40-46 (1979); EP 117,060; and EP
117,058.

Suitable host cells for cloning or expressing the DNA in the
25 vectors herein include prokaryote, yeast, or higher eukaryote cells.
Suitable prokaryotes for this purpose include but are not limited to
eubacteria, such as Gram-negative or Gram-positive organisms, for
example, *Enterobacteriaceae* such as *Escherichia*, e.g., *E. coli*,
Enterobacter, *Erwinia*, *Klebsiella*, *Proteus*, *Salmonella*, e.g.,
30 *Salmonella typhimurium*, *Serratia*, e.g., *Serratia marcescans*, and
Shigella, as well as *Bacilli* such as *B. subtilis* and *B. licheniformis*
(e.g., *B. licheniformis* 41P disclosed in DD 266,710 published 12
April 1989), *Pseudomonas* such as *P. aeruginosa*, and *Streptomyces*.
Preferably, the host cell should secrete minimal amounts of
35 proteolytic enzymes.

In addition to prokaryotes, eukaryotic microbes such as
filamentous fungi or yeast are suitable cloning or expression hosts
for vectors. Suitable host cells for the expression of glycosylated

polypeptide are derived from multicellular organisms. Examples of all such host cells are described further in WO97/25428.

Host cells are transfected and preferably transformed with the above-described expression or cloning vectors and cultured in nutrient media modified as appropriate for inducing promoters, selecting transformants, or amplifying the genes encoding the desired sequences.

Transfection refers to the taking up of an expression vector by a host cell whether or not any coding sequences are in fact expressed. Numerous methods of transfection are known to the ordinarily skilled artisan, for example, CaPO$_4$ and electroporation. Successful transfection is generally recognized when any indication of the operation of this vector occurs within the host cell.

Transformation means introducing DNA into an organism so that the DNA is replicable, either as an extrachromosomal element or by chromosomal integrant. Depending on the host cell used, transformation is done using standard techniques appropriate to such cells. The calcium treatment employing calcium chloride, as described in Sambrook et al., supra, or electroporation is generally used for prokaryotes or other cells that contain substantial cell-wall barriers. Infection with Agrobacterium tumefaciens is used for transformation of certain plant cells, as described by Shaw et al., Gene, 23:315 (1983) and WO 89/05859 published 29 June 1989. In addition, plants may be transfected using ultrasound treatment as described in WO 91/00358 published 10 January 1991.

For mammalian cells without such cell walls, the calcium phosphate precipitation method of Graham and van der Eb, Virology, 52:456-457 (1978) may be employed. General aspects of mammalian cell host system transformations have been described in U.S. Pat. No. 4,399,216. Transformations into yeast are typically carried out according to the method of Van Solingen et al., J. Bact., 130:946 (1977) and Hsiao et al., Proc. Natl. Acad. Sci. (USA), 76:3829 (1979). However, other methods for introducing DNA into cells, such as by nuclear microinjection, electroporation, bacterial protoplast fusion with intact cells, or polycations, e.g., polybrene, polyornithine, may also be used. For various techniques for transforming mammalian cells, see Keown et al., Methods in Enzymology, 185:527-537 (1990) and Mansour et al., Nature, 336:348-352 (1988).

Prokaryotic cells may be cultured in suitable culture media as described generally in Sambrook et al., _supra_. Examples of commercially available culture media include Ham's F10 (Sigma), Minimal Essential Medium ("MEM", Sigma), RPMI-1640 (Sigma), and

5   Dulbecco's Modified Eagle's Medium ("DMEM", Sigma). Any such media may be supplemented as necessary with hormones and/or other growth factors (such as insulin, transferrin, or epidermal growth factor), salts (such as sodium chloride, calcium, magnesium, and phosphate), buffers (such as HEPES), nucleosides (such as adenosine and

10 thymidine), antibiotics (such as Gentamycin™ drug), trace elements (defined as inorganic compounds usually present at final concentrations in the micromolar range), and glucose or an equivalent energy source. Any other necessary supplements may also be included at appropriate concentrations that would be known to those skilled in

15 the art. The culture conditions, such as temperature, pH, and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

In general, principles, protocols, and practical techniques for maximizing the productivity of mammalian cell cultures can be found

20 in _Mammalian Cell Biotechnology: A Practical Approach_, M. Butler, ed. (IRL Press, 1991).

The expressed polypeptides may be recovered from the culture medium as a secreted polypeptide, although may also may be recovered from host cell lysates when directly produced without a secretory

25 signal. If the polypeptide is membrane-bound, it can be released from the membrane using a suitable detergent solution (e.g. Triton-X 100) or its extracellular region may be released by enzymatic cleavage.

When the polypeptide is produced in a recombinant cell other than one of human origin, it is free of proteins or polypeptides of

30 human origin. However, it is usually necessary to recover or purify the polypeptide from recombinant cell proteins or polypeptides to obtain preparations that are substantially homogeneous. As a first step, the culture medium or lysate may be centrifuged to remove particulate cell debris. The following are procedures exemplary of

35 suitable purification procedures: by fractionation on an ion-exchange column; ethanol precipitation; reverse phase HPLC; chromatography on silica or on a cation-exchange resin such as DEAE; chromatofocusing; SDS-PAGE; ammonium sulfate precipitation; gel filtration using, for example, Sephadex G-75; and protein A Sepharose columns to remove

40 contaminants such as IgG.

TACI receptor variants and BCMA receptor variants are
contemplated for use in the invention. Such variants can be prepared
using any suitable technique in the art.   The receptor variants can
be prepared by introducing appropriate nucleotide changes into the

5   receptor DNA, and/or by synthesis of the desired receptor
polypeptide. Those skilled in the art will appreciate that amino
acid changes may alter post-translational processes of the receptor,
such as changing the number or position of glycosylation sites or
altering the membrane anchoring characteristics.

10   Variations in the native sequence receptor or in various domains
of the receptors described herein, can be made, for example, using
any of the techniques and guidelines for conservative and non-
conservative mutations set forth, for instance, in U.S. Patent No.
5,364,934. Variations may be a substitution, deletion or insertion

15   of one or more codons encoding the receptor that results in a change
in the amino acid sequence of the receptor as compared with the
native sequence receptor. Optionally the variation is by
substitution of at least one amino acid with any other amino acid in
one or more of the domains of the receptor. Guidance in determining

20   which amino acid residue may be inserted, substituted or deleted
without adversely affecting the desired activity may be found by
comparing the sequence of the receptor with that of homologous known
protein molecules and minimizing the number of amino acid sequence
changes made in regions of high homology. Amino acid substitutions

25   can be the result of replacing one amino acid with another amino acid
having similar structural and/or chemical properties, such as the
replacement of a leucine with a serine, i.e., conservative amino acid
replacements. Insertions or deletions may optionally be in the range
of about 1 to 5 amino acids. The variation allowed may be determined

30   by systematically making insertions, deletions or substitutions of
amino acids in the sequence and testing the resulting variants for
activity exhibited by the full-length or mature native sequence.

TACI or BCMA polypeptide fragments are provided herein. Such
fragments may be truncated at the N-terminus or C-terminus, or may

35   lack internal residues, for example, when compared with a full-length
native protein. Certain fragments lack amino acid residues that are
not essential for a desired biological activity of the receptor
polypeptide. Optionally, the TACI or BCMA polypeptide fragments
comprise ECD deletion variants in which one or more amino acid

40   residues have been deleted from the N-terminus or C-terminus of the

52

receptor ECD sequence. Preferably, such ECD deletion variants have
at least one biological activity as compared to the native receptor
sequence.

5   TACI or BCMA fragments may be prepared by any of a number of
conventional techniques. Desired peptide fragments may be chemically
synthesized. An alternative approach involves generating receptor
fragments by enzymatic digestion, e.g., by treating the protein with
an enzyme known to cleave proteins at sites defined by particular
amino acid residues, or by digesting the DNA with suitable
10   restriction enzymes and isolating the desired fragment. Yet another
suitable technique involves isolating and amplifying a DNA fragment
encoding a desired polypeptide fragment, by polymerase chain reaction
(PCR). Oligonucleotides that define the desired termini of the DNA
fragment are employed at the 5' and 3' primers in the PCR.

15   In particular embodiments, conservative substitutions of
interest are shown in Table 1 under the heading of preferred
substitutions. If such substitutions result in a change in
biological activity, then more substantial changes, denominated
exemplary substitutions in Table 1, or as further described below in
20   reference to amino acid classes, are introduced and the products
screened.

Table 1

Original Residue	Exemplary Substitutions	Preferred Substitutions
Ala (A)	val; leu; ile	val
Arg (R)	lys; gln; asn	lys
Asn (N)	gln; his; lys; arg	gln
Asp (D)	glu	glu
Cys (C)	ser	ser
Gln (Q)	asn	asn
Glu (E)	asp	asp
Gly (G)	pro; ala	ala
His (H)	asn; gln; lys; arg	arg
Ile (I)	leu; val; met; ala; phe; norleucine	leu
Leu (L)	norleucine; ile; val; met; ala; phe	ile
Lys (K)	arg; gln; asn	arg
Met (M)	leu; phe; ile	leu
Phe (F)	leu; val; ile; ala; tyr	leu
Pro (P)	ala	ala
Ser (S)	thr	thr
Thr (T)	ser	ser
Trp (W)	tyr; phe	tyr
Tyr (Y)	trp; phe; thr; ser	phe
Val (V)	ile; leu; met; phe; ala; norleucine	leu

Substantial modifications in function or immunological identity of the receptor polypeptide are accomplished by selecting substitutions that differ significantly in their effect on

5   maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues are divided into groups based on common side-chain properties:

10   (1) hydrophobic: norleucine, met, ala, val, leu, ile;

(2) neutral hydrophilic: cys, ser, thr;

(3) acidic: asp, glu;

(4) basic: asn, gln, his, lys, arg;

(5) residues that influence chain orientation: gly, pro; and

15   (6) aromatic: trp, tyr, phe.

Non-conservative substitutions will entail exchanging a member of one of these classes for another class. Such substituted residues also may be introduced into the conservative substitution sites or,

20   more preferably, into the remaining (non-conserved) sites.

The variations can be made using methods known in the art such as oligonucleotide-mediated (site-directed) mutagenesis, alanine scanning, and PCR mutagenesis. Site-directed mutagenesis [Carter et al., Nucl. Acids Res., 13:4331 (1986); Zoller et al., Nucl. Acids

25   Res., 10:6487 (1987)], cassette mutagenesis [Wells et al., Gene, 34:315 (1985)], restriction selection mutagenesis [Wells et al., Philos. Trans. R. Soc. London SerA, 317:415 (1986)] or other known techniques can be performed on the cloned DNA to produce the receptor variant DNA.

30   Scanning amino acid analysis can also be employed to identify one or more amino acids along a contiguous sequence. Among the preferred scanning amino acids are relatively small, neutral amino acids. Such amino acids include alanine, glycine, serine, and cysteine. Alanine is typically a preferred scanning amino acid among

35   this group because it eliminates the side-chain beyond the beta-carbon and is less likely to alter the main-chain conformation of the variant [Cunningham and Wells, Science, 244: 1081-1085 (1989)]. Alanine is also typically preferred because it is the most common amino acid. Further, it is frequently found in both buried and

40   exposed positions [Creighton, The Proteins, (W.H. Freeman & Co.,

N.Y.); Chothia, J. Mol. Biol., 150:1 (1976)]. If alanine
substitution does not yield adequate amounts of variant, an isoteric
amino acid can be used.

5   Soluble forms of TACI receptors or BCMA receptors may also be
employed as antagonists in the methods of the invention. Such
soluble forms of TACI or BCMA may comprise or consist of
extracellular domains of the respective receptor (and lacking
transmembrane and intracellular domains of the respective receptor).
The extracellular domain sequences themselves of TACI or BCMA may be
10   used as antagonists, or may be further modified as described below
(such as by fusing to an immunoglobulin, epitope tag or leucine
zipper). Certain extracellular domain regions of TACI and BCMA have
been described in the literature and may be further delineated using
techniques known to the skilled artisan. Those skilled in the art
15   will be able to select, without undue experimentation, a desired
extracellular domain sequence of either TACI or BCMA to employ as an
antagonist.

          Immunoadhesin molecules are further contemplated for use in the
methods herein. TACI receptor immunoadhesins may comprise various
20   forms of TACI, such as the full length polypeptide as well as soluble
forms of the receptor which comprise an extracellular domain (ECD)
sequence or a fragment of the ECD sequence. In one embodiment, the
molecule may comprise a fusion of the TACI receptor with an
immunoglobulin or a particular region of an immunoglobulin. For a
25   bivalent form of the immunoadhesin, such a fusion could be to the Fc
region of an IgG molecule. The Ig fusions preferably include the
substitution of a soluble (transmembrane domain deleted or
inactivated) form of the receptor polypeptide in place of at least
one variable region within an Ig molecule. In a particularly
30   preferred embodiment, the immunoglobulin fusion includes the hinge,
CH2 and CH3, or the hinge, CH1, CH2 and CH3 regions of an IgG1
molecule. For the production of immunoglobulin fusions, see also US
Patent No. 5,428,130 issued June 27, 1995 and Chamow et al., TIBTECH,
14:52-60 (1996).

35   The simplest and most straightforward immunoadhesin design
combines the binding domain(s) of the adhesin (e.g. the extracellular
domain (ECD) of a receptor) with the Fc region of an immunoglobulin
heavy chain. Ordinarily, when preparing the immunoadhesins of the
present invention, nucleic acid encoding the binding domain of the
40   adhesin will be fused C-terminally to nucleic acid encoding the N-

                                    55

terminus of an immunoglobulin constant domain sequence, however N-terminal fusions are also possible.

Typically, in such fusions the encoded chimeric polypeptide will retain at least functionally active hinge, C_H2 and C_H3 domains of the
5   constant region of an immunoglobulin heavy chain. Fusions are also made to the C-terminus of the Fc portion of a constant domain, or immediately N-terminal to the C_H1 of the heavy chain or the corresponding region of the light chain. The precise site at which the fusion is made is not critical; particular sites are well known
10 and may be selected in order to optimize the biological activity, secretion, or binding characteristics of the immunoadhesin.

In a preferred embodiment, the adhesin sequence is fused to the N-terminus of the Fc region of immunoglobulin G_1 (IgG$_1$). It is possible to fuse the entire heavy chain constant region to the
15 adhesin sequence. However, more preferably, a sequence beginning in the hinge region just upstream of the papain cleavage site which defines IgG Fc chemically (i.e. residue 216, taking the first residue of heavy chain constant region to be 114), or analogous sites of other immunoglobulins is used in the fusion. In a particularly
20 preferred embodiment, the adhesin amino acid sequence is fused to (a) the hinge region and C_H2 and C_H3 or (b) the C_H1, hinge, C_H2 and C_H3 domains, of an IgG heavy chain.

For bispecific immunoadhesins, the immunoadhesins are assembled as multimers, and particularly as heterodimers or heterotetramers.
25 Generally, these assembled immunoglobulins will have known unit structures. A basic four chain structural unit is the form in which IgG, IgD, and IgE exist. A four chain unit is repeated in the higher molecular weight immunoglobulins; IgM generally exists as a pentamer of four basic units held together by disulfide bonds. IgA globulin,
30 and occasionally IgG globulin, may also exist in multimeric form in serum. In the case of multimer, each of the four units may be the same or different.

Various exemplary assembled immunoadhesins within the scope herein are schematically diagrammed below:
35   (a) AC_L-AC_L;
        (b) $AC_H-(AC_H,\ AC_L-AC_H,\ AC_L-V_HC_H,\ or\ V_LC_L-AC_H)$;
        (c) $AC_L-AC_H-(AC_L-AC_H,\ AC_L-V_HC_H,\ V_LC_L-AC_H,\ or\ V_LC_L-V_HC_H)$
        (d) $AC_L-V_HC_H-(AC_H,\ or\ AC_L-V_HC_H,\ or\ V_LC_L-AC_H)$;
        (e) $V_LC_L-AC_H-(AC_L-V_HC_H,\ or\ V_LC_L-AC_H)$; and
40   (f) $(A-Y)_n-(V_LC_L-V_HC_H)_2$,

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wherein each A represents identical or different adhesin amino acid sequences;

V_L is an immunoglobulin light chain variable domain;

V_H is an immunoglobulin heavy chain variable domain;

5   C_L is an immunoglobulin light chain constant domain;

C_H is an immunoglobulin heavy chain constant domain;

n is an integer greater than 1;

Y designates the residue of a covalent cross-linking agent.

In the interests of brevity, the foregoing structures only show
10   key features; they do not indicate joining (J) or other domains of the immunoglobulins, nor are disulfide bonds shown. However, where such domains are required for binding activity, they shall be constructed to be present in the ordinary locations which they occupy in the immunoglobulin molecules.

15   Alternatively, the adhesin sequences can be inserted between immunoglobulin heavy chain and light chain sequences, such that an immunoglobulin comprising a chimeric heavy chain is obtained. In this embodiment, the adhesin sequences are fused to the 3' end of an immunoglobulin heavy chain in each arm of an immunoglobulin, either
20   between the hinge and the C_H2 domain, or between the C_H2 and C_H3 domains. Similar constructs have been reported by Hoogenboom et al., Mol. Immunol., 28:1027-1037 (1991).

Although the presence of an immunoglobulin light chain is not required in the immunoadhesins of the present invention, an
25   immunoglobulin light chain might be present either covalently associated to an adhesin-immunoglobulin heavy chain fusion polypeptide, or directly fused to the adhesin. In the former case, DNA encoding an immunoglobulin light chain is typically coexpressed with the DNA encoding the adhesin-immunoglobulin heavy chain fusion
30   protein. Upon secretion, the hybrid heavy chain and the light chain will be covalently associated to provide an immunoglobulin-like structure comprising two disulfide-linked immunoglobulin heavy chain-light chain pairs. Methods suitable for the preparation of such structures are, for example, disclosed in U.S. Patent No. 4,816,567,
35   issued 28 March 1989.

Immunoadhesins are most conveniently constructed by fusing the cDNA sequence encoding the adhesin portion in-frame to an immunoglobulin cDNA sequence. However, fusion to genomic immunoglobulin fragments can also be used (see, e.g. Aruffo et al.,
40   Cell, 61:1303-1313 (1990); and Stamenkovic et al., Cell, 66:1133-1144

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(1991)). The latter type of fusion requires the presence of Ig
regulatory sequences for expression. cDNAs encoding IgG heavy-chain
constant regions can be isolated based on published sequences from
cDNA libraries derived from spleen or peripheral blood lymphocytes,
by hybridization or by polymerase chain reaction (PCR) techniques.
The cDNAs encoding the "adhesin" and the immunoglobulin parts of the
immunoadhesin are inserted in tandem into a plasmid vector that
directs efficient expression in the chosen host cells.

        Examples of such soluble ECD sequences include polypeptides
comprising amino acids 2-166 of the TACI sequence shown in Figure 1,
and described further in the Examples below. The TACI receptor
immunoadhesin can be made according to any of the methods described
in the art.

        BCMA receptor immunoadhesins can be similarly constructed.
Examples of soluble ECD sequences for use in constructing BCMA
immunoadhesins may include polypeptides comprising amino acids 5-51
of the BCMA sequence shown in Figure 2, and described further in the
Examples below.

        In another embodiment, the TACI or BCMA receptor may be
covalently modified by linking the receptor polypeptide to one of a
variety of nonproteinaceous polymers, e.g., polyethylene glycol
(PEG), polypropylene glycol, or polyoxyalkylenes, in the manner set
forth in U.S. Patent Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417;
4,791,192 or 4,179,337. Such pegylated forms of the TACI or BCMA
receptor may be prepared using techniques known in the art.

        Leucine zipper forms of these molecules are also contemplated by
the invention. "Leucine zipper" is a term in the art used to refer
to a leucine rich sequence that enhances, promotes, or drives
dimerization or trimerization of its fusion partner (e.g., the
sequence or molecule to which the leucine zipper is fused or linked
to). Various leucine zipper polypeptides have been described in the
art. See, e.g., Landschulz et al., Science, 240:1759 (1988); US
Patent 5,716,805; WO 94/10308; Hoppe et al., FEBS Letters, 344:1991
(1994); Maniatis et al., Nature, 341:24 (1989). Those skilled in the
art will appreciate that a leucine zipper sequence may be fused at
either the 5' or 3' end of the TACI or BCMA receptor molecule.

        The TACI or BCMA polypeptides of the present invention may also
be modified in a way to form chimeric molecules by fusing the
receptor polypeptide to another, heterologous polypeptide or amino
acid sequence. Preferably, such heterologous polypeptide or amino

acid sequence is one which acts to oligimerize the chimeric molecule.
In one embodiment, such a chimeric molecule comprises a fusion of the
TACI or BCMA receptor polypeptide with a tag polypeptide which
provides an epitope to which an anti-tag antibody can selectively

5   bind. The epitope tag is generally placed at the amino- or carboxyl-
terminus of the receptor polypeptide. The presence of such epitope-
tagged forms of the receptor can be detected using an antibody
against the tag polypeptide. Also, provision of the epitope tag
enables the receptor to be readily purified by affinity purification

10   using an anti-tag antibody or another type of affinity matrix that
binds to the epitope tag. Various tag polypeptides and their
respective antibodies are well known in the art. Examples include
poly-histidine (poly-his) or poly-histidine-glycine (poly-his-gly)
tags; the flu HA tag polypeptide and its antibody 12CA5 [Field et

15   al., Mol. Cell. Biol., 8:2159-2165 (1988)]; the c-myc tag and the
8F9, 3C7, 6E10, G4, B7 and 9E10 antibodies thereto [Evan et al.,
Molecular and Cellular Biology, 5:3610-3616 (1985)]; and the Herpes
Simplex virus glycoprotein D (gD) tag and its antibody [Paborsky et
al., Protein Engineering, 3(6):547-553 (1990)]. Other tag

20   polypeptides include the Flag-peptide [Hopp et al., BioTechnology,
6:1204-1210 (1988)]; the KT3 epitope peptide [Martin et al., Science,
255:192-194 (1992)]; an α-tubulin epitope peptide [Skinner et al., J.
Biol. Chem., 266:15163-15166 (1991)]; and the T7 gene 10 protein
peptide tag [Lutz-Freyermuth et al., Proc. Natl. Acad. Sci. USA,

25   87:6393-6397 (1990)].

It is contemplated that anti-TACI receptor antibodies, anti-BCMA
receptor antibodies, anti-TALL-1 antibodies, or anti-APRIL antibodies
may also be employed in the presently disclosed methods. Examples of
such molecules include neutralizing or blocking antibodies which can

30   preferably inhibit binding of TALL-1 or APRIL to the TACI or to the
BCMA receptors. The anti-TACI antibodies, anti-BCMA, anti-TALL-1, or
anti-APRIL antibodies may be monoclonal antibodies. Monoclonal
antibodies may be prepared using hybridoma methods, such as those
described by Kohler and Milstein, Nature, 256:495 (1975). In a

35   hybridoma method, a mouse, hamster, or other appropriate host animal,
is typically immunized with an immunizing agent to elicit lymphocytes
that produce or are capable of producing antibodies that will
specifically bind to the immunizing agent. Alternatively, the
lymphocytes may be immunized in vitro.

The immunizing agent will typically include the TACI or BCMA
polypeptide, or TALL-1 or APRIL polypeptide, or a fusion protein
thereof. Generally, either peripheral blood lymphocytes ("PBLs") are
used if cells of human origin are desired, or spleen cells or lymph
5   node cells are used if non-human mammalian sources are desired. The
lymphocytes are then fused with an immortalized cell line using a
suitable fusing agent, such as polyethylene glycol, to form a
hybridoma cell [Goding, Monoclonal Antibodies: Principles and
Practice, Academic Press, (1986) pp. 59-103]. Immortalized cell
10 lines are usually transformed mammalian cells, particularly myeloma
cells of rodent, bovine and human origin. Usually, rat or mouse
myeloma cell lines are employed. The hybridoma cells may be cultured
in a suitable culture medium that preferably contains one or more
substances that inhibit the growth or survival of the unfused,
15 immortalized cells. For example, if the parental cells lack the
enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or
HPRT), the culture medium for the hybridomas typically will include
hypoxanthine, aminopterin, and thymidine ("HAT medium"), which
substances prevent the growth of HGPRT-deficient cells.
20   Preferred immortalized cell lines are those that fuse
efficiently, support stable high level expression of antibody by the
selected antibody-producing cells, and are sensitive to a medium such
as HAT medium. More preferred immortalized cell lines are murine
myeloma lines, which can be obtained, for instance, from the Salk
25 Institute Cell Distribution Center, San Diego, California and the
American Type Culture Collection, Manassas, Virginia. Human myeloma
and mouse-human heteromyeloma cell lines also have been described for
the production of human monoclonal antibodies [Kozbor, J. Immunol.,
133:3001 (1984); Brodeur et al., Monoclonal Antibody Production
30 Techniques and Applications, Marcel Dekker, Inc., New York, (1987)
pp. 51-63].
        The culture medium in which the hybridoma cells are cultured can
then be assayed for the presence of monoclonal antibodies directed
against TACI, BCMA, TALL-1 or APRIL. Preferably, the binding
35 specificity of monoclonal antibodies produced by the hybridoma cells
is determined by immunoprecipitation or by an in vitro binding assay,
such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay
(ELISA). Such techniques and assays are known in the art. The
binding affinity of the monoclonal antibody can, for example, be
40 determined by the Scatchard analysis of Munson and Pollard, Anal.

Biochem., 107:220 (1980). Optionally, the anti-TACI, anti-BCMA, anti-TALL-1, or anti-APRIL antibodies will have a binding affinity of at least 10nM, preferably, of at least 5nM, and more preferably, of at least 1nM for the respective receptor or ligand, as determined in

5   a binding assay.

After the desired hybridoma cells are identified, the clones may be subcloned by limiting dilution procedures and grown by standard methods [Goding, supra]. Suitable culture media for this purpose include, for example, Dulbecco's Modified Eagle's Medium and RPMI-

10   1640 medium. Alternatively, the hybridoma cells may be grown in vivo as ascites in a mammal.

The monoclonal antibodies secreted by the subclones may be isolated or purified from the culture medium or ascites fluid by conventional immunoglobulin purification procedures such as, for

15   example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

The monoclonal antibodies may also be made by recombinant DNA methods, such as those described in U.S. Patent No. 4,816,567. DNA encoding the monoclonal antibodies of the invention can be readily

20   isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells of the invention serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors,

25   which are then transfected into host cells such as simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. The DNA also may be modified, for example, by substituting the coding sequence for

30   human heavy and light chain constant domains in place of the homologous murine sequences [U.S. Patent No. 4,816,567; Morrison et al., supra] or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. Such a non-immunoglobulin polypeptide can be

35   substituted for the constant domains of an antibody of the invention, or can be substituted for the variable domains of one antigen-combining site of an antibody of the invention to create a chimeric bivalent antibody.

The antibodies may be monovalent antibodies. Methods for

40   preparing monovalent antibodies are well known in the art. For

example, one method involves recombinant expression of immunoglobulin light chain and modified heavy chain. The heavy chain is truncated generally at any point in the Fc region so as to prevent heavy chain crosslinking. Alternatively, the relevant cysteine residues are

5   substituted with another amino acid residue or are deleted so as to prevent crosslinking.

In vitro methods are also suitable for preparing monovalent antibodies. Digestion of antibodies to produce fragments thereof, particularly, Fab fragments, can be accomplished using routine

10 techniques known in the art.

In a further embodiment, antibodies or antibody fragments can be isolated from antibody phage libraries generated using the techniques described in McCafferty et al., Nature, 348:552-554 (1990). Clackson et al., Nature, 352:624-628 (1991) and Marks et al., J. Mol. Biol.,

15 222:581-597 (1991) describe the isolation of murine and human antibodies, respectively, using phage libraries. Subsequent publications describe the production of high affinity (nM range) human antibodies by chain shuffling (Marks et al., Bio/Technology, 10:779-783 (1992)), as well as combinatorial infection and in vivo

20 recombination as a strategy for constructing very large phage libraries (Waterhouse et al., Nuc. Acids. Res., 21:2265-2266 (1993)). Thus, these techniques are viable alternatives to traditional monoclonal antibody hybridoma techniques for isolation of monoclonal antibodies.

25 The DNA also may be modified, for example, by substituting the coding sequence for human heavy- and light-chain constant domains in place of the homologous murine sequences (U.S. Patent No. 4,816,567; Morrison, et al., Proc. Natl Acad. Sci. USA, 81:6851 (1984)), or by covalently joining to the immunoglobulin coding sequence all or part

30 of the coding sequence for a non-immunoglobulin polypeptide.

Typically such non-immunoglobulin polypeptides are substituted for the constant domains of an antibody, or they are substituted for the variable domains of one antigen-combining site of an antibody to create a chimeric bivalent antibody comprising one antigen-combining

35 site having specificity for an antigen and another antigen-combining site having specificity for a different antigen.

A humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which

40 are typically taken from an "import" variable domain. Humanization

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can be essentially performed following the method of Winter and co-
workers (Jones et al., Nature, 321:522-525 (1986); Riechmann et al.,
Nature, 332:323-327 (1988); Verhoeyen et al., Science, 239:1534-1536
(1988)), by substituting rodent CDRs or CDR sequences for the

5   corresponding sequences of a human antibody.   Accordingly, such
"humanized" antibodies are chimeric antibodies (U.S. Patent No.
4,816,567) wherein substantially less than an intact human variable
domain has been substituted by the corresponding sequence from a non-
human species.   In practice, humanized antibodies are typically human

10   antibodies in which some CDR residues and possibly some FR residues
are   substituted   by   residues   from   analogous   sites   in   rodent
antibodies.

The choice of human variable domains, both light and heavy, to
be used in making the humanized antibodies is very important to

15   reduce antigenicity.   According to the so-called "best-fit" method,
the sequence of the variable domain of a rodent antibody is screened
against the entire library of known human variable-domain sequences.
The human sequence which is closest to that of the rodent is then
accepted as the human framework (FR) for the humanized antibody (Sims

20   et al., J. Immunol., 151:2296 (1993); Chothia et al., J. Mol. Biol.,
196:901 (1987)).   Another method uses a particular framework derived
from the consensus sequence of all human antibodies of a particular
subgroup of light or heavy chains.   The same framework may be used
for several different humanized antibodies (Carter et al., Proc.

25   Natl. Acad. Sci. USA, 89:4285 (1992); Presta et al., J. Immnol.,
151:2623 (1993)).

It is further important that antibodies be humanized with
retention of high affinity for the antigen and other favorable
biological properties.   To achieve this goal, according to a

30   preferred method, humanized antibodies are prepared by a process of
analysis of the parental sequences and various conceptual humanized
products using three-dimensional models of the parental and humanized
sequences.   Three-dimensional immunoglobulin models are commonly
available and are familiar to those skilled in the art.   Computer

35   programs are available which illustrate and display probable three-
dimensional   conformational   structures   of   selected   candidate
immunoglobulin sequences.   Inspection of these displays permits
analysis of the likely role of the residues in the functioning of the
candidate immunoglobulin sequence, i.e., the analysis of residues

40   that influence the ability of the candidate immunoglobulin to bind

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its antigen. In this way, FR residues can be selected and combined from the recipient and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the CDR residues are directly and most substantially involved in influencing antigen binding.

Alternatively, it is now possible to produce transgenic animals (e.g., mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy-chain joining region (J_H) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. See, e.g., Jakobovits et al., Proc. Natl. Acad. Sci. USA, 90:2551 (1993); Jakobovits et al., Nature, 362:255-258 (1993); Bruggermann et al., Year in Immuno., 7:33 (1993); and Duchosal et al., Nature, 355:258 (1992). Human antibodies can also be derived from phage-display libraries (Hoogenboom et al., J. Mol. Biol., 227:381 (1991); Marks et al., J. Mol. Biol., 222:581-597 (1991); Vaughan et al., Nature Biotech, 14:309 (1996)).

As described in the Examples below, particular anti-APRIL antibodies have been prepared. Four of these antibodies, 3C6.4.2, 5G8.2.2, 5E8.7.4, and 5E11.1.2, have been deposited with ATCC, and have been assigned accession numbers PTA-1347, PTA-1345, PTA-1344 and PTA-1346. In one embodiment, the anti-APRIL antibodies disclosed herein will have the same biological characteristics as the monoclonal antibodies secreted by the hybridoma cell lines deposited under accession numbers PTA-1347, PTA-1345, PTA-1344 or PTA-1346. The term "biological characteristics" is used to refer to the in vitro and/or in vivo activities or properties of the monoclonal antibody, such as the ability to bind to APRIL or to substantially block or reduce TACI or BCMA binding or activation by APRIL. Optionally, the anti-APRIL monoclonal antibody will have the same blocking activity as the 3C6.4.2 antibody, as determined by its ability to block binding of APRIL to TACI or BCMA. Optionally, the anti-APRIL monoclonal antibody will bind to the same epitope as the 5G8.2.2 antibody, the 5E8.7.4 antibody, the 3C6.4.2 antibody or the 5E11.1.2 antibody disclosed in the Examples below. Such epitope binding property can be determined for instance in a competitive

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inhibition binding assay, which techniques are known in the art. Such an anti-APRIL antibody will preferably competitively inhibit binding of either the 5G8.2.2 antibody, the 5E8.7.4 antibody, the 3C6.4.2 antibody or the 5E11.1.2 antibody to APRIL. It is contemplated that chimeric or humanized anti-APRIL antibodies can be constructed (such as by using the techniques described above) using or incorporating selected fragments or domain sequences from any of the afore-mentioned deposited anti-APRIL antibodies.

B.   ASSAY METHODS

Ligand/receptor binding studies may be carried out in any known assay method, such as competitive binding assays, direct and indirect sandwich assays, and immunoprecipitation assays. Cell-based assays and animal models can be used to further understand the interaction between the ligands and receptors identified herein and the development and pathogenesis of the conditions and diseases referred to herein.

In one approach, mammalian cells may be transfected with the ligands or receptors described herein, and the ability of the agonists or antagonists to stimulate or inhibit binding or activity is analyzed. Suitable cells can be transfected with the desired gene, and monitored for activity. Such transfected cell lines can then be used to test the ability of antagonist(s) or agonist(s) to inhibit or stimulate, for example, to modulate B-cell proliferation or Ig secretion. Cells transfected with the coding sequence of the genes identified herein can further be used to identify drug candidates for the treatment of immune related diseases or cancer.

In addition, primary cultures derived from transgenic animals can be used in the cell-based assays. Techniques to derive continuous cell lines from transgenic animals are well known in the art. [see, e.g., Small et al., Mol. Cell. Biol., 5:642-648 (1985)].

One suitable cell based assay is the addition of epitope-tagged ligand (e.g., AP or Flag) to cells that have or express the respective receptor, and analysis of binding (in presence or absence or prospective antagonists) by FACS staining with anti-tag antibody. In another assay, the ability of an antagonist to inhibit the TALL-1 or APRIL induced proliferation of B cells is assayed. B cells or cell lines are cultured with TALL-1 or APRIL in the presence or absence or prospective antagonists and the proliferation of B cells can be measured by ^{3}H-thymidine incorporation or cell number.

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The results of the cell based *in vitro* assays can be further
verified using *in vivo* animal models. A variety of well known animal
models can be used to further understand the role of the agonists and
antagonists identified herein in the development and pathogenesis of

5   for instance, immune related disease or cancer, and to test the
efficacy of the candidate therapeutic agents. The *in vivo* nature of
such models makes them particularly predictive of responses in human
patients. Animal models of immune related diseases include both non-
recombinant and recombinant (transgenic) animals. Non-recombinant

10   animal models include, for example, rodent, e.g., murine models.
Such models can be generated by introducing cells into syngeneic
mice using standard techniques, e.g. subcutaneous injection, tail
vein injection, spleen implantation, intraperitoneal implantation,
and implantation under the renal capsule.

15   Animal models, for example, for graft-versus-host disease are
known. Graft-versus-host disease occurs when immunocompetent cells
are transplanted into immunosuppressed or tolerant patients. The
donor cells recognize and respond to host antigens. The response can
vary from life threatening severe inflammation to mild cases of

20   diarrhea and weight loss. Graft-versus-host disease models provide a
means of assessing T cell reactivity against MHC antigens and minor
transplant antigens. A suitable procedure is described in detail in
Current Protocols in Immunology, unit 4.3.

An animal model for skin allograft rejection is a means of

25   testing the ability of T cells to mediate *in vivo* tissue destruction
which is indicative of and a measure of their role in anti-viral and
tumor immunity. The most common and accepted models use murine tail-
skin grafts. Repeated experiments have shown that skin allograft
rejection is mediated by T cells, helper T cells and killer-effector

30   T cells, and not antibodies. [Auchincloss, H. Jr. and Sachs, D.
H., Fundamental Immunology, 2nd ed., W. E. Paul ed., Raven Press, NY,
1989, 889-992]. A suitable procedure is described in detail in
Current Protocols in Immunology, unit 4.4. Other transplant
rejection models which can be used to test the compositions of the

35   invention are the allogeneic heart transplant models described by
Tanabe, M. et al., Transplantation, (1994) 58:23 and Tinubu, S. A. et
al., J. Immunol., (1994) 4330-4338.

Animal models for delayed type hypersensitivity provides an
assay of cell mediated immune function as well. Delayed type

40   hypersensitivity reactions are a T cell mediated in vivo immune

response characterized by inflammation which does not reach a peak
until after a period of time has elapsed after challenge with an
antigen. These reactions also occur in tissue specific autoimmune
diseases such as multiple sclerosis (MS) and experimental autoimmune

5   encephalomyelitis (EAE, a model for MS). A suitable procedure is
described in detail in Current Protocols in Immunology, unit 4.5.

An animal model for arthritis is collagen-induced arthritis.
This model shares clinical, histological and immunological
characteristics of human autoimmune rheumatoid arthritis and is an

10   acceptable model for human autoimmune arthritis. Mouse and rat
models are characterized by synovitis, erosion of cartilage and
subchondral bone. The compounds of the invention can be tested for
activity against autoimmune arthritis using the protocols described
in Current Protocols in Immunology, above, units 15.5. See also the

15   model using a monoclonal antibody to CD18 and VLA-4 integrins
described in Issekutz, A. C. et al., Immunology, (1996) 88:569.
Example 10 below further describes a CIA murine model.

A model of asthma has been described in which antigen-induced
airway hyper-reactivity, pulmonary eosinophilia and inflammation are

20   induced by sensitizing an animal with ovalbumin and then challenging
the animal with the same protein delivered by aerosol. Several
animal models (guinea pig, rat, non-human primate) show symptoms
similar to atopic asthma in humans upon challenge with aerosol
antigens. Murine models have many of the features of human asthma.

25   Suitable procedures to test the compositions of the invention for
activity and effectiveness in the treatment of asthma are described
by Wolyniec, W. W. et al., Am. J. Respir. Cell Mol. Biol., (1998)
18:777 and the references cited therein.

Additionally, the compositions of the invention can be tested on

30   animal models for psoriasis like diseases. The compounds of the
invention can be tested in the scid/scid mouse model described by
Schon, M. P. et al., Nat. Med., (1997) 3:183, in which the mice
demonstrate histopathologic skin lesions resembling psoriasis.
Another suitable model is the human skin/scid mouse chimera prepared

35   as described by Nickoloff, B. J. et al., Am. J. Path., (1995)
146:580.

Various animal models are well known for testing anti-cancer
activity of a candidate therapeutic composition. These include human
tumor xenografting into athymic nude mice or scid/scid mice, or

40   genetic murine tumor models such as p53 knockout mice.

Recombinant (transgenic) animal models can be engineered by
introducing the coding portion of the molecules identified herein
into the genome of animals of interest, using standard techniques for
producing transgenic animals.   Animals that can serve as a target
5   for transgenic manipulation include, without limitation, mice, rats,
rabbits, guinea pigs, sheep, goats, pigs, and non-human primates,
e.g. baboons, chimpanzees and monkeys.   Techniques known in the art
to introduce a transgene into such animals include pronucleic
microinjection (Hoppe and Wanger, U.S. Patent No. 4,873,191);
10   retrovirus-mediated gene transfer into germ lines (e.g., Van der
Putten et al., Proc. Natl. Acad. Sci. USA, 82, 6148-615 [1985]); gene
targeting in embryonic stem cells (Thompson et al., Cell, 56, 313-321
[1989]); electroporation of embryos (Lo, Mol. Cel. Biol., 3, 1803-
1814 [1983]); sperm-mediated gene transfer (Lavitrano et al., Cell,
15   57, 717-73 [1989]).   For review, see, for example, U.S. Patent No.
4,736,866.

For the purpose of the present invention, transgenic animals
include those that carry the transgene only in part of their cells
("mosaic animals").   The transgene can be integrated either as a
20   single transgene, or in concatamers, e.g., head-to-head or head-to-
tail tandems.   Selective introduction of a transgene into a
particular cell type is also possible by following, for example, the
technique of Lasko et al., Proc. Natl. Acad. Sci. USA, 89, 6232-636
(1992).

25   The expression of the transgene in transgenic animals can be
monitored by standard techniques.   For example, Southern blot
analysis or PCR amplification can be used to verify the integration
of the transgene.   The level of mRNA expression can then be analyzed
using techniques such as in situ hybridization, Northern blot
30   analysis, PCR, or immunocytochemistry.   The animals may be further
examined for signs of immune disease pathology, for example by
histological examination to determine infiltration of immune cells
into specific tissues or for the presence of cancerous or malignant
tissue.

35   Alternatively, "knock out" animals can be constructed which have
a defective or altered gene encoding a polypeptide identified herein,
as a result of homologous recombination between the endogenous gene
encoding the polypeptide and altered genomic DNA encoding the same
polypeptide introduced into an embryonic cell of the animal.   For

example, cDNA encoding a particular polypeptide can be used to clone genomic DNA encoding that polypeptide in accordance with established techniques. A portion of the genomic DNA encoding a particular polypeptide can be deleted or replaced with another gene, such as a

5   gene encoding a selectable marker which can be used to monitor integration. Typically, several kilobases of unaltered flanking DNA (both at the 5' and 3' ends) are included in the vector [see e.g., Thomas and Capecchi, Cell, 51:503 (1987) for a description of homologous recombination vectors]. The vector is introduced into an

10 embryonic stem cell line (e.g., by electroporation) and cells in which the introduced DNA has homologously recombined with the endogenous DNA are selected [see e.g., Li et al., Cell, 69:915 (1992)]. The selected cells are then injected into a blastocyst of an animal (e.g., a mouse or rat) to form aggregation chimeras [see

15 e.g., Bradley, in Teratocarcinomas and Embryonic Stem Cells: A Practical Approach, E. J. Robertson, ed. (IRL, Oxford, 1987), pp. 113-152]. A chimeric embryo can then be implanted into a suitable pseudopregnant female foster animal and the embryo brought to term to create a "knock out" animal. Progeny harboring the homologously

20 recombined DNA in their germ cells can be identified by standard techniques and used to breed animals in which all cells of the animal contain the homologously recombined DNA. Knockout animals can be characterized for instance, for their ability to defend against certain pathological conditions and for their development of

25 pathological conditions due to absence of the polypeptide.

        C.   FORMULATIONS
        The antagonists or agonists described herein are preferably employed in a carrier. Suitable carriers and their formulations are

30 described in Remington's Pharmaceutical Sciences, 16th ed., 1980, Mack Publishing Co., edited by Oslo et al. Typically, an appropriate amount of a pharmaceutically-acceptable salt is used in the carrier to render the formulation isotonic. Examples of the carrier include saline, Ringer's solution and dextrose solution. The pH of the solution is

35 preferably from about 5 to about 8, and more preferably from about 7.4 to about 7.8. It will be apparent to those persons skilled in the art that certain carriers may be more preferable depending upon, for instance, the route of administration and concentration of agent being administered. The carrier may be in the form of a lyophilized

40 formulation or aqueous solution.

Acceptable carriers, excipients, or stabilizers are preferably nontoxic to cells and/or recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG).

The formulation may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other.

The antagonist or agonist may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences 16th edition, Oslo, A. Ed. (1980).

The formulations to be used for *in vivo* administration should be sterile. This is readily accomplished by filtration through sterile filtration membranes.

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, *e.g.* films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No.

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3,773,919), copolymers of L-glutamic acid and γ ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and

5   leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods.

10   D.   MODES OF THERAPY
         The antagonist or agonist molecules described herein are useful in treating various pathological conditions, such as immune related diseases or cancer. These conditions can be treated by stimulating or inhibiting a selected activity associated with TALL-1, APRIL, TACI

15   or BCMA in a mammal through administration of one or more antagonists or agonists of the invention.
         Diagnosis in mammals of the various pathological conditions described herein can be made by the skilled practitioner. Diagnostic techniques are available in the art which allow, e.g., for the

20   diagnosis or detection of cancer or immune related disease in a mammal. For instance, cancers may be identified through techniques, including but not limited to, palpation, blood analysis, x-ray, NMR and the like. Immune related diseases can also be readily identified. In systemic lupus erythematosus, the central mediator of

25   disease is the production of auto-reactive antibodies to self proteins/tissues and the subsequent generation of immune-mediated inflammation. Multiple organs and systems are affected clinically including kidney, lung, musculoskeletal system, mucocutaneous, eye, central nervous system, cardiovascular system, gastrointestinal

30   tract, bone marrow and blood.
         Rheumatoid arthritis (RA) is a chronic systemic autoimmune inflammatory disease that mainly involves the synovial membrane of multiple joints with resultant injury to the articular cartilage. The pathogenesis is T lymphocyte dependent and is associated with the

35   production of rheumatoid factors, auto-antibodies directed against self IgG, with the resultant formation of immune complexes that attain high levels in joint fluid and blood. These complexes in the joint may induce the marked infiltrate of lymphocytes and monocytes into the synovium and subsequent marked synovial changes; the joint

40   space/fluid if infiltrated by similar cells with the addition of

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numerous neutrophils. Tissues affected are primarily the joints,
often in symmetrical pattern. However, extra-articular disease also
occurs in two major forms. One form is the development of extra-
articular lesions with ongoing progressive joint disease and typical
5   lesions of pulmonary fibrosis, vasculitis, and cutaneous ulcers. The
second form of extra-articular disease is the so called Felty's
syndrome which occurs late in the RA disease course, sometimes after
joint disease has become quiescent, and involves the presence of
neutropenia, thrombocytopenia and splenomegaly. This can be
10   accompanied by vasculitis in multiple organs with formations of
infarcts, skin ulcers and gangrene. Patients often also develop
rheumatoid nodules in the subcutis tissue overlying affected joints;
the nodules late stage have necrotic centers surrounded by a mixed
inflammatory cell infiltrate. Other manifestations which can occur
15   in RA include: pericarditis, pleuritis, coronary arteritis,
intestitial pneumonitis with pulmonary fibrosis, keratoconjunctivitis
sicca, and rhematoid nodules.
        Juvenile chronic arthritis is a chronic idiopathic inflammatory
disease which begins often at less than 16 years of age. Its
20   phenotype has some similarities to RA; some patients which are
rhematoid factor positive are classified as juvenile rheumatoid
arthritis. The disease is sub-classified into three major
categories: pauciarticular, polyarticular, and systemic. The
arthritis can be severe and is typically destructive and leads to
25   joint ankylosis and retarded growth. Other manifestations can
include chronic anterior uveitis and systemic amyloidosis.
        Spondyloarthropathies are a group of disorders with some common
clinical features and the common association with the expression of
HLA-B27 gene product. The disorders include: ankylosing sponylitis,
30   Reiter's syndrome (reactive arthritis), arthritis associated with
inflammatory bowel disease, spondylitis associated with psoriasis,
juvenile onset spondyloarthropathy and undifferentiated
spondyloarthropathy. Distinguishing features include sacroileitis
with or without spondylitis; inflammatory asymmetric arthritis;
35   association with HLA-B27 (a serologically defined allele of the HLA-B
locus of class I MHC); ocular inflammation, and absence of
autoantibodies associated with other rheumatoid disease. The cell
most implicated as key to induction of the disease is the CD8+ T
lymphocyte, a cell which targets antigen presented by class I MHC
40   molecules. CD8+ T cells may react against the class I MHC allele

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HLA-B27 as if it were a foreign peptide expressed by MHC class I
molecules. It has been hypothesized that an epitope of HLA-B27 may
mimic a bacterial or other microbial antigenic epitope and thus
induce a CD8+ T cells response.

5   Systemic sclerosis (scleroderma) has an unknown etiology. A
hallmark of the disease is induration of the skin; likely this is
induced by an active inflammatory process. Scleroderma can be
localized or systemic; vascular lesions are common and endothelial
cell injury in the microvasculature is an early and important event
10   in the development of systemic sclerosis; the vascular injury may be
immune mediated. An immunologic basis is implied by the presence of
mononuclear cell infiltrates in the cutaneous lesions and the
presence of anti-nuclear antibodies in many patients. ICAM-1 is
often upregulated on the cell surface of fibroblasts in skin lesions
15   suggesting that T cell interaction with these cells may have a role
in the pathogenesis of the disease. Other organs involved include:
the gastrointestinal tract: smooth muscle atrophy and fibrosis
resulting in abnormal peristalsis/motility; kidney: concentric
subendothelial intimal proliferation affecting small arcuate and
20   interlobular arteries with resultant reduced renal cortical blood
flow, results in proteinuria, azotemia and hypertension; skeletal
muscle: atrophy, interstitial fibrosis; inflammation; lung:
interstitial pneumonitis and interstitial fibrosis; and heart:
contraction band necrosis, scarring/fibrosis.

25   Idiopathic inflammatory myopathies including dermatomyositis,
polymyositis and others are disorders of chronic muscle inflammation
of unknown etiology resulting in muscle weakness. Muscle
injury/inflammation is often symmetric and progressive.
Autoantibodies are associated with most forms. These myositis-
30   specific autoantibodies are directed against and inhibit the function
of components, proteins and RNA's, involved in protein synthesis.

           Sjogren's syndrome is due to immune-mediated inflammation and
subsequent functional destruction of the tear glands and salivary
glands. The disease can be associated with or accompanied by
35   inflammatory connective tissue diseases. The disease is associated
with autoantibody production against Ro and La antigens, both of
which are small RNA-protein complexes. Lesions result in
keratoconjunctivitis sicca, xerostomia, with other manifestations or
associations including bilary cirrhosis, peripheral or sensory
40   neuropathy, and palpable purpura.

Systemic vasculitis are diseases in which the primary lesion is inflammation and subsequent damage to blood vessels which results in ischemia/necrosis/degeneration to tissues supplied by the affected vessels and eventual end-organ dysfunction in some cases.

5   Vasculitides can also occur as a secondary lesion or sequelae to other immune-inflammatory mediated diseases such as rheumatoid arthritis, systemic sclerosis, etc., particularly in diseases also associated with the formation of immune complexes. Diseases in the primary systemic vasculitis group include: systemic necrotizing

10   vasculitis: polyarteritis nodosa, allergic angiitis and granulomatosis, polyangiitis; Wegener's granulomatosis; lymphomatoid granulomatosis; and giant cell arteritis. Miscellaneous vasculitides include: mucocutaneous lymph node syndrome (MLNS or Kawasaki's disease), isolated CNS vasculitis, Behet's disease, thromboangiitis

15   obliterans (Buerger's disease) .and cutaneous necrotizing venulitis. The pathogenic mechanism of most of the types of vasculitis listed is believed to be primarily due to the deposition of immunoglobulin complexes in the vessel wall and subsequent induction of an inflammatory response either via ADCC, complement activation, or

20   both.

Sarcoidosis is a condition of unknown etiology which is characterized by the presence of epithelioid granulomas in nearly any tissue in the body; involvement of the lung is most common. The pathogenesis involves the persistence of activated macrophages and

25   lymphoid cells at sites of the disease with subsequent chronic sequelae resultant from the release of locally and systemically active products released by these cell types.

Autoimmune hemolytic anemia including autoimmune hemolytic anemia, immune pancytopenia, and paroxysmal noctural hemoglobinuria

30   is a result of production of antibodies that react with antigens expressed on the surface of red blood cells (and in some cases other blood cells including platelets as well) and is a reflection of the removal of those antibody coated cells via complement mediated lysis and/or ADCC/Fc-receptor-mediated mechanisms.

35   In autoimmune thrombocytopenia including thrombocytopenic purpura, and immune-mediated thrombocytopenia in other clinical settings, platelet destruction/removal occurs as a result of either antibody or complement attaching to platelets and subsequent removal by complement lysis, ADCC or FC-receptor mediated mechanisms.

Thyroiditis including Grave's disease, Hashimoto's thyroiditis, juvenile lymphocytic thyroiditis, and atrophic thyroiditis, are the result of an autoimmune response against thyroid antigens with production of antibodies that react with proteins present in and
5   often specific for the thyroid gland. Experimental models exist including spontaneous models: rats (BUF and BB rats) and chickens (obese chicken strain); inducible models: immunization of animals with either thyroglobulin, thyroid microsomal antigen (thyroid peroxidase).

10   Type I diabetes mellitus or insulin-dependent diabetes is the autoimmune destruction of pancreatic islet β cells; this destruction is mediated by auto-antibodies and auto-reactive T cells. Antibodies to insulin or the insulin receptor can also produce the phenotype of insulin-non-responsiveness.

15   Immune mediated renal diseases, including glomerulonephritis and tubulointerstitial nephritis, are the result of antibody or T lymphocyte mediated injury to renal tissue either directly as a result of the production of autoreactive antibodies or T cells against renal antigens or indirectly as a result of the deposition of
20   antibodies and/or immune complexes in the kidney that are reactive against other, non-renal antigens. Thus other immune-mediated diseases that result in the formation of immune-complexes can also induce immune mediated renal disease as an indirect sequelae. Both direct and indirect immune mechanisms result in inflammatory response
25   that produces/induces lesion development in renal tissues with resultant organ function impairment and in some cases progression to renal failure. Both humoral and cellular immune mechanisms can be involved in the pathogenesis of lesions.

Demyelinating diseases of the central and peripheral nervous
30   systems, including Multiple Sclerosis; idiopathic demyelinating polyneuropathy or Guillain-Barr syndrome; and Chronic Inflammatory Demyelinating Polyneuropathy, are believed to have an autoimmune basis and result in nerve demyelination as a result of damage caused to oligodendrocytes or to myelin directly. In MS there is evidence
35   to suggest that disease induction and progression is dependent on T lymphocytes. Multiple Sclerosis is a demyelinating disease that is T lymphocyte-dependent and has either a relapsing-remitting course or a chronic progressive course. The etiology is unknown; however, viral infections, genetic predisposition, environment, and autoimmunity all
40   contribute. Lesions contain infiltrates of predominantly T

lymphocyte mediated, microglial cells and infiltrating macrophages; CD4+T lymphocytes are the predominant cell type at lesions. The mechanism of oligodendrocyte cell death and subsequent demyelination is not known but is likely T lymphocyte driven.

Inflammatory and Fibrotic Lung Disease, including Eosinophilic Pneumonias; Idiopathic Pulmonary Fibrosis, and Hypersensitivity Pneumonitis may involve a disregulated immune-inflammatory response. Inhibition of that response would be of therapeutic benefit.

Autoimmune or Immune-mediated Skin Disease including Bullous Skin Diseases, Erythema Multiforme, and Contact Dermatitis are mediated by auto-antibodies, the genesis of which is T lymphocyte-dependent.

Psoriasis is a T lymphocyte-mediated inflammatory disease. Lesions contain infiltrates of T lymphocytes, macrophages and antigen processing cells, and some neutrophils.

Allergic diseases, including asthma; allergic rhinitis; atopic dermatitis; food hypersensitivity; and urticaria are T lymphocyte dependent. These diseases are predominantly mediated by T lymphocyte induced inflammation, IgE mediated-inflammation or a combination of both.

Transplantation associated diseases, including Graft rejection and Graft-Versus-Host-Disease (GVHD) are T lymphocyte-dependent; inhibition of T lymphocyte function is ameliorative.

Other diseases in which intervention of the immune and/or inflammatory response have benefit are Infectious disease including but not limited to viral infection (including but not limited to AIDS, hepatitis A, B, C, D, E) bacterial infection, fungal infections, and protozoal and parasitic infections (molecules (or derivatives/agonists) which stimulate the MLR can be utilized therapeutically to enhance the immune response to infectious agents), diseases of immunodeficiency (molecules/derivatives/agonists) which stimulate the MLR can be utilized therapeutically to enhance the immune response for conditions of inherited, acquired, infectious induced (as in HIV infection), or iatrogenic (i.e. as from chemotherapy) immunodeficiency), and neoplasia.

The antagonist(s) or agonist(s) can be administered in accord with known methods, such as intravenous administration as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerebrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes.

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Optionally, administration may be performed through mini-pump infusion using various commercially available devices. The antagonists or agonists may also be employed using gene therapy techniques which have been described in the art.

Effective dosages and schedules for administering antagonists or agonists may be determined empirically, and making such determinations is within the skill in the art. Single or multiple dosages may be employed. It is presently believed that an effective dosage or amount of antagonist or agonist used alone may range from about 1 µg/kg to about 100 mg/kg of body weight or more per day. Interspecies scaling of dosages can be performed in a manner known in the art, e.g., as disclosed in Mordenti et al., Pharmaceut. Res., 8:1351 (1991).

When in vivo administration of an agonist or antagonist thereof is employed, normal dosage amounts may vary from about 10 ng/kg to up to 100 mg/kg of mammal body weight or more per day, preferably about 1 µg/kg/day to 10 mg/kg/day, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature; see, for example, U.S. Pat. Nos. 4,657,760; 5,206,344; or. 5,225,212. It is anticipated that different formulations will be effective for different treatment compounds and different disorders, that administration targeting one organ or tissue, for example, may necessitate delivery in a manner different from that to another organ or tissue. Those skilled in the art will understand that the dosage of antagonist or agonist that must be administered will vary depending on, for example, the mammal which will receive the agonist or antagonist, the route of administration, and other drugs or therapies being administered to the mammal.

Depending on the type of cells and/or severity of the disease, about 1 µg/kg to 15 mg/kg (e.g. 0.1-20 mg/kg) of antagonist antibody or agonist antibody is an initial candidate dosage for administration, whether, for example, by one or more separate administrations, or by continuous infusion. A typical daily dosage might range from about 1 µg/kg to 100 mg/kg or more, depending on the factors mentioned above. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of disease symptoms occurs. However, other dosage regimens may be useful.

Optionally, prior to administration of any antagonist or agonist, the mammal or patient can be tested to determine levels or

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activity of TALL-1 or APRIL, or TACI or BCMA. Such testing may be conducted by ELISA or FACS of serum samples or peripheral blood leukocytes.

5   A single type of antagonist or agonist may be used in the methods of the invention. For example, a TALL-1 antagonist, such as a TACI receptor immunoadhesin molecule, may be administered. Alternatively, the skilled practitioner may opt to employ a combination of antagonists or agonists in the methods, e.g., a combination of a TACI receptor immunoadhesin and an anti-APRIL antibody. It may further be desirable
10   to employ a dual antagonist, i.e., an antagonist which acts to block or inhibit both TALL-1 and APRIL. Such an antagonist molecule may, for instance, bind to epitopes conserved between TALL-1 and APRIL, or TACI and BCMA.

           It is contemplated that yet additional therapies may be employed
15   in the methods. The one or more other therapies may include but are not limited to, administration of radiation therapy, cytokine(s), growth inhibitory agent(s), chemotherapeutic agent(s), cytotoxic agent(s), tyrosine kinase inhibitors, ras farnesyl transferase inhibitors, angiogenesis inhibitors, and cyclin-dependent kinase
20   inhibitors which are known in the art and defined further with particularity in Section I above. In addition, therapies based on therapeutic antibodies that target tumor antigens such as Rituxan™ or Herceptin™ as well as anti-angiogenic antibodies such as anti-VEGF.

           Preparation and dosing schedules for chemotherapeutic agents may
25   be used according to manufacturers' instructions or as determined empirically by the skilled practitioner. Preparation and dosing schedules for such chemotherapy are also described in *Chemotherapy Service* Ed., M.C. Perry, Williams & Wilkins, Baltimore, MD (1992). The chemotherapeutic agent may precede, or follow administration of,
30   e.g. an antagonist, or may be given simultaneously therewith. The antagonist may also be combined with an anti-oestrogen compound such as tamoxifen or an anti-progesterone such as onapristone (see, EP 616812) in dosages known for such molecules.

           It may be desirable to also administer antibodies against other
35   antigens, such as antibodies which bind to CD20, CD11a, CD18, CD40, ErbB2, EGFR, ErbB3, ErbB4, or vascular endothelial factor (VEGF). Alternatively, or in addition, two or more antibodies binding the same or two or more different antigens disclosed herein may be co-administered to the patient. Sometimes, it may be beneficial to also
40   administer one or more cytokines to the patient. In one embodiment,

the antagonists herein are co-administered with a growth inhibitory
agent.   For example, the growth inhibitory agent may be administered
first, followed by an antagonist of the present invention.

The antagonist or agonist (and one or more other therapies) may
5   be administered concurrently or sequentially.   Following administration
of antagonist or agonist, treated cells *in vitro* can be analyzed.
Where there has been *in vivo* treatment, a treated mammal can be
monitored in various ways well known to the skilled practitioner.   For
instance, markers of B cell activity such as Ig production (non-
10   specific or antigen specific) can be assayed.

III.   METHODS OF SCREENING
The invention also encompasses methods of screening molecules to
identify those which can act as agonists or antagonists of the
15   APRIL/TACI/BCMA interaction or the TALL-1/TACI/BCMA interaction.
Such molecules may comprise small molecules or polypeptides,
including antibodies. Examples of small molecules include, but are
not limited to, small peptides or peptide-like molecules, preferably
soluble peptides, and synthetic non-peptidyl organic or inorganic
20   compounds.   The screening assays for drug candidates are designed to
identify compounds or molecules that bind or complex with the ligand
or receptor polypeptides identified herein, or otherwise interfere
with the interaction of these polypeptides with other cellular
proteins.   Such screening assays will include assays amenable to
25   high-throughput screening of chemical libraries, making them
particularly suitable for identifying small molecule drug candidates.

The assays can be performed in a variety of formats, including
protein-protein binding assays, biochemical screening assays,
immunoassays, and cell-based assays, which are well characterized in
30   the art.

Assays for, for instance, antagonists are common in that they
call for contacting the drug candidate with a ligand or receptor
polypeptide identified herein under conditions and for a time
sufficient to allow these two components to interact.

35   In binding assays, the interaction is binding and the complex
formed can be isolated or detected in the reaction mixture.   In a
particular embodiment, the ligand or receptor polypeptide identified
herein or the drug candidate is immobilized on a solid phase, e.g.,
on a microtiter plate, by covalent or non-covalent attachments.   Non-
40   covalent attachment generally is accomplished by coating the solid

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surface with a solution of the ligand or receptor polypeptide and

drying. Alternatively, an immobilized antibody, e.g., a monoclonal

antibody, specific for the ligand or receptor polypeptide to be

immobilized can be used to anchor it to a solid surface. The assay

5   is performed by adding the non-immobilized component, which may be

labeled by a detectable label, to the immobilized component, e.g.,

the coated surface containing the anchored component. When the

reaction is complete, the non-reacted components are removed, e.g.,

by washing, and complexes anchored on the solid surface are detected.

10   When the originally non-immobilized component carries a detectable

label, the detection of label immobilized on the surface indicates

that complexing occurred. Where the originally non-immobilized

component does not carry a label, complexing can be detected, for

example, by using a labeled antibody specifically binding the

15   immobilized complex.

        If the candidate compound interacts with but does not bind to a

particular ligand or receptor polypeptide identified herein, its

interaction with that polypeptide can be assayed by methods well

known for detecting protein-protein interactions. Such assays

20   include traditional approaches, such as, e.g., cross-linking, co-

immunoprecipitation, and co-purification through gradients or

chromatographic columns. In addition, protein-protein interactions

can be monitored by using a yeast-based genetic system described by

Fields and co-workers (Fields and Song, Nature (London), 340:245-246

25   (1989); Chien et al., Proc. Natl. Acad. Sci. USA, 88:9578-9582

(1991)) as disclosed by Chevray and Nathans, Proc. Natl. Acad. Sci.

USA, 89: 5789-5793 (1991). Many transcriptional activators, such as

yeast GAL4, consist of two physically discrete modular domains, one

acting as the DNA-binding domain, the other one functioning as the

30   transcription-activation domain. The yeast expression system

described in the foregoing publications (generally referred to as the

"two-hybrid system") takes advantage of this property, and employs

two hybrid proteins, one in which the target protein is fused to the

DNA-binding domain of GAL4, and another, in which candidate

35   activating proteins are fused to the activation domain. The

expression of a GAL1-lacZ reporter gene under control of a GAL4-

activated promoter depends on reconstitution of GAL4 activity via

protein-protein interaction. Colonies containing interacting

polypeptides are detected with a chromogenic substrate for β-

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galactosidase. A complete kit (MATCHMAKER™) for identifying protein-
protein interactions between two specific proteins using the two-
hybrid technique is commercially available from Clontech. This
system can also be extended to map protein domains involved in
5   specific protein interactions as well as to pinpoint amino acid
residues that are crucial for these interactions.

Compounds or molecules that interfere with the interaction of a
ligand or receptor polypeptide identified herein and other intra- or
extracellular components can be tested as follows: usually a
10   reaction mixture is prepared containing the product of the gene and
the intra- or extracellular component under conditions and for a time
allowing for the interaction and binding of the two products. To
test the ability of a candidate compound to inhibit binding, the
reaction is run in the absence and in the presence of the test
15   compound. In addition, a placebo may be added to a third reaction
mixture, to serve as positive control. The binding (complex
formation) between the test compound and the intra- or extracellular
component present in the mixture is monitored as described
hereinabove. The formation of a complex in the control reaction(s)
20   but not in the reaction mixture containing the test compound
indicates that the test compound interferes with the interaction of
the test compound and its reaction partner.

To assay for antagonists, the ligand or receptor polypeptide may
be added to a cell along with the compound to be screened for a
25   particular activity and the ability of the compound to inhibit the
activity of interest in the presence of the ligand or receptor
polypeptide indicates that the compound is an antagonist to the
ligand or receptor polypeptide. Alternatively, antagonists may be
detected by combining the ligand or receptor polypeptide and a
30   potential antagonist with membrane-bound polypeptide receptors or
recombinant receptors under appropriate conditions for a competitive
inhibition assay. The ligand or receptor polypeptide can be labeled,
such as by radioactivity, such that the number of polypeptide
molecules bound to the receptor can be used to determine the
35   effectiveness of the potential antagonist. The gene encoding the
receptor can be identified by numerous methods known to those of
skill in the art, for example, ligand panning and FACS sorting.
Coligan et al., Current Protocols in Immun., 1(2): Chapter 5 (1991).
Preferably, expression cloning is employed wherein polyadenylated RNA
40   is prepared from a cell responsive to the ligand or receptor

polypeptide and a cDNA library created from this RNA is divided into
pools and used to transfect COS cells or other cells that are not
responsive to the ligand or receptor polypeptide. Transfected cells
that are grown on glass slides are exposed to labeled ligand or
5   receptor polypeptide. The ligand or receptor polypeptide can be
labeled by a variety of means including iodination or inclusion of a
recognition site for a site-specific protein kinase. Following
fixation and incubation, the slides are subjected to autoradiographic
analysis. Positive pools are identified and sub-pools are prepared
10   and re-transfected using an interactive sub-pooling and re-screening
process, eventually yielding a single clone that encodes the putative
receptor.
        As an alternative approach, labeled ligand polypeptide can be
photoaffinity-linked with cell membrane or extract preparations that
15   express receptor molecule. Cross-linked material is resolved by PAGE
and exposed to X-ray film. The labeled complex containing the
receptor can be excised, resolved into peptide fragments, and
subjected to protein micro-sequencing. The amino acid sequence
obtained from micro- sequencing would be used to design a set of
20   degenerate oligonucleotide probes to screen a cDNA library to
identify the gene encoding the putative receptor.

        IV.   ARTICLES OF MANUFACTURE
        In another embodiment of the invention, an article of
25   manufacture containing materials useful for the treatment of the
disorders described above is provided. The article of manufacture
comprises a container and a label. Suitable containers include, for
example, bottles, vials, syringes, and test tubes. The containers
may be formed from a variety of materials such as glass or plastic.
30   The container holds a composition which is effective for treating the
condition and may have a sterile access port (for example the
container may be an intravenous solution bag or a vial having a
stopper pierceable by a hypodermic injection needle). The active
agents in the composition may comprise TALL-1 antagonist(s) or APRIL
35   antagonist(s), or TACI agonist(s) or BCMA agonist(s). The label on,
or associated with, the container indicates that the composition is
used for treating the condition of choice. The article of
manufacture may further comprise a second container comprising a
pharmaceutically-acceptable buffer, such as phosphate-buffered
40   saline, Ringer's solution and dextrose solution. It may further

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include other materials desirable from a commercial and user
standpoint, including other buffers, diluents, filters, needles,
syringes, and package inserts with instructions for use.

The following examples are offered by way of illustration and
5   not by way of limitation. The disclosures of all citations in the
specification are expressly incorporated herein by reference.

EXAMPLE 1

Identification and Expression Cloning of TACI, A Receptor
10   for TALL-1 Ligand

A chimeric protein, referred to as "AP-TALL-1", was prepared
using human placenta alkaline phosphatase (AP) fused to the N-
terminus of a TALL-1 polypeptide consisting of amino acids 136-285
15   shown in Figure 3. The AP was obtained by PCR amplification using
pAPtag-5 (Genehunter Corporation) as a template, and fused and cloned
into the expression vector, pCMV-1 Flag (Sigma), with AP at the N-
terminus of TALL-1. The AP-TALL-1 was transiently transfected (using
Lipofectamine reagent; Gibco-BRL) and expressed in human embryonic
20   kidney 293 cells (ATCC). AP-TNF-alpha (Pennica et al., infra) was
similarly prepared. AP-EDA (comprising amino acids 241-391 of EDA;
Srivastava et al., Proc. Natl. Acad. Sci., 94:13069-13074 (1997)) was
also similarly prepared. The conditioned medium from the transfected
293 cells was filtered (0.45 micron), stored at 4°C in a buffer
25   containing 20mM Hepes (pH 7.0) and 1 mM sodium azide, and used for
subsequent cell staining procedures.

In addition, an N-terminal Flag-tagged form of TALL-1 was
constructed in a pCMV-1 Flag vector. To promote the trimerization of
this Flag-tagged TALL-1 construct, a trimeric form of leucine-zipper
30   sequence [Science, 262:1401-1407 (1993)] was inserted between the
Flag-tag and the TALL-1 (consisting of amino acids 136-285 of Figure
3), and this construct was referred to as "Flag-LZP-TALL-1". The
Flag-LZP-TALL-1 was purified using M2-agarose gel (Sigma) from serum-
free medium of 293 cells transfected with the Flag-LZP-TALL-1
35   expressing plasmid.

AP reactivity could be readily detected when AP-TALL-1
conditioned medium, but not control AP conditioned medium, was
applied to IM-9 cells (ATCC) which have been shown to exhibit high
levels of TALL-1 binding activity (data not shown). The purified
40   Flag-LZP-TALL-1 also bound to the IM-9 cells, as determined by FACS

analysis (Data not shown). Importantly, the binding of AP-TALL-1 to
IM-9 cells was effectively blocked by preincubation with purified
Flag-LZP-TALL-1 but not with purified TNF-alpha [prepared essentially
as described in Pennica et al., Nature, 312:724-729 (1984)],

5   suggesting that both forms of TALL-1 were functional and the
respective binding of AP-TALL-1 and Flag-LZP-TALL-1 to IM-9 cells was
specific.

       To identify a receptor for TALL-1, a cDNA expression library was
constructed in pRK5 vector (EP 307,247, published March 15, 1989)

10   using PolyA+.mRNA derived from the IM-9 cells [Flanagan et al., Cell,
63:185 (1990)]. Pools of ~1000 cDNA clones (Miniprep DNA (Qiagen))
from the library were transfected (using Lipofectamine) into COS 7
cells (ATCC) in 6 well plates, which after 36-48 hours, were then
incubated with AP-TALL-1 conditioned medium, washed, and stained for

15   AP activity in situ. A positive pool was broken down to successively
smaller size pools. After three rounds·of screening, a single cDNA
encoding an AP-TALL-1 binding activity was identified. Sequencing of
the cDNA insert revealed a single open reading frame predicted to
encode a protein of 265 amino acids. This 265 amino acid polypeptide

20   (referred to as "hTACI (265)" in Figure 6), when aligned with the
TACI sequence shown in Fig. 1 (referred to as "hTACI" in Figure 6),
revealed a high percentage of sequence identity, particularly in the
ECD. The alignment of these two TACI sequences is shown in Figure 6.
It is believed that the 265 amino acid form of TACI may be a spliced

25   variant of the TACI sequence shown in Figure 1.

       In an in vitro assay, COS 7 cells (ATCC) were seeded into 12
well plates 24 hours before transfection. The cells were then
transfected with 1 microgram TACI (the 265 amino acid form of human
TACI described above, cloned in pRK5B vector, infra) or vector

30   plasmid (pRK5B) alone. 18-24 hours after transfection, the cells
were incubated with conditioned medium containing AP-TALL-1; AP-TNF-
alpha; or AP-EDA for 1 hour at room temperature and stained for AP
activity in situ as described in Tartaglia et al., Cell, 83:1263-1271
(1995).

35   As shown in Fig. 7, AP-TALL-1 (Fig. 7A) but not AP-TNF-alpha
(Fig. 7B) or AP-EDA (Fig. 7C) was found to bind COS 7 cells
transfected with TACI. The AP-TALL-1 did not stain cells transfected
with vector plasmid alone (Fig. 7D). AP-TALL-1 binding to the TACI
transfected COS 7 cells was effectively blocked by a Flag-tagged form

of TALL-1, but not by a Flag-tagged form of LIGHT (Mauri et al.,
supra], another TNF homolog (data not shown).

EXAMPLE 2

5   ### Binding of TALL-1 or APRIL to TACI-IgG and BCMA-IgG

Flag-tagged ligands were prepared as follows. Amino acids 82-
240 of LIGHT (Mauri et al., Immunity, 8:21-30 (1998)) were subcloned
into pCMV-1 Flag (Sigma) using a NotI site to fuse amino acids 1-27
of the Flag signal and tag sequence upstream of the LIGHT sequence.

10   Amino acids 105-250 of APRIL (see Fig. 4) were similarly cloned into
pCMV-1 Flag (Sigma), except that a HindIII site was used, resulting
in fusion to amino acids 1-24 of the Flag signal and tag sequence.
Amino acids 124-285 of TALL-1 (see Fig. 3) were fused to amino acids
1-27 of the Flag signal and tag sequence, as described above for

15   Flag-LIGHT. AP-APRIL was prepared by cloning amino acids 105-250 of
APRIL (see Fig. 4) into a pCMV-1 Flag vector encoding human placental
alkaline phosphatase such that the APRIL encoding sequence was fused
C-terminally to AP, while the AP was fused C-terminally to Flag. AP-
TALL-1 was prepared by cloning amino acids 135-285 of TALL-1 (see

20   Fig. 3) into the pCMV-1 Flag, AP vector, as described above for AP-
APRIL. The respective tagged proteins were then expressed in 293
cells or CHO cells and purified using M2 anti-Flag resin (Sigma).

One µg of the purified human or Flag-LIGHT (control), Flag-
APRIL, or Flag-TALL-1, or Flag-AP-APRIL, or Flag-AP-TALL-1 was

25   incubated with 1 µg of purified human immunoadhesin containing the
IgG1-Fc fusion of the ECD of DcR3 (control; Pitti et al., Nature,
396:699-703 (1998)) or TACI or BCMA overnight at 4° C in duplicate.
The TACI-ECD.hFc immunoadhesins were prepared by methods described in
Ashkenazi et al., Proc. Natl. Acad. Sci., 88:10535-10539 (1991). The

30   immunoadhesin constructs consisted of amino acids 2-166 of the human
TACI polypeptide (see Figure 1). The TACI-ECD constructs were
expressed in CHO cells using a heterologous signal sequence (pre-pro
trypsin amino acids 1-17 of pCMV-1 Flag (Sigma)) and encoding the
human IgG1 Fc region downstream of the TACI sequence, and then

35   purified by protein A affinity chromatography. The BCMA-ECD
immunoadhesins were prepared by methods described in Ashkenazi et
al., as cited above. The immunoadhesin constructs consisted of amino
acids 5-51 of the human BCMA polypeptide (see Figure 2). The BCMA-
ECD constructs were expressed in CHO cells using a heterologous

signal sequence (pre-pro trypsin amino acids 1-17 of pCMV-1 Flag

(Sigma)) and encoding the human IgG1 Fc region downstream of the BCMA

sequence, and then purified by protein A affinity chromatography.

One set of reactions (Figure 8; Panels A-C) was subjected to

5   immunoprecipitation through the receptor-immunoadhesin with protein

A-agarose (Repligen). The second set of reactions (Figure 8; panels

D-F) was subjected to immunoprecipitation through the Flag-tagged

ligand with Anti-Flag mAb-M2-agarose (Sigma). The immunoprecipitates

were then analyzed by Western blot with horseradish peroxidase-

10   conjugated anti-Flag M2 mAb (Sigma) to detect the Flag-tagged ligands

(Figures 8A-C) or horseradish peroxidase-conjugated goat anti-human

IgG pAb (Amersham) to detect the receptor-immunoadhesins (Figures 8D-

F).

The data shows that Flag-LIGHT bound to DcR3-IgG, but not to

15   TACI-IgG or BCMA-IgG. Flag-APRIL and Flag-AP-APRIL bound to TACI-IgG

and BCMA-IgG, but not to DcR3-IgG. Similarly, Flag-TALL-1 and Flag-

AP-TALL-1 bound to TACI-IgG and BCMA-IgG, but not to DcR3-IgG. These

assay results indicated that APRIL and TALL-1 can each bind in a

specific and stable manner to TACI and to BCMA.

20   In a similarly conducted co-immunoprecipitation assay, TACI-Fc

(described in Example 2), HVEM-Fc (Montgomery et al., supra); DR3-Fc

(Chinnaiyan et al., Science, 274:990 (1996); Marsters et al., Curr.

Biol., 6:1669 (1996)); or DR6-Fc (Pan et al., FEBS Letters, 431:351-

356 (1998))(1 µg/ml) was incubated with Flag-TALL-1 (1 µg/ml;

25   prepared as described in Example 2). One set of reactions was

subjected to immunoprecipitation through the receptor-Fc fusion with

protein A Agarose; the second set of reactions was subjected to

immunoprecipitation through the ligand with anti-Flag antibody. The

samples were analyzed by Western blot, as above. Flag-TALL-1 was not

30   detected in anti-Fc co-immunoprecipitation with the Fc fusion

constructs of HVEM, DR3, or DR6 (Figure 8G). Conversely, TACI-Fc was

not detected in anti-Flag co-immunoprecipitations with HVEM-Fc, DR3-

Fc, or DR6-Fc (Figure 8H).

Additional assays were conducted to determine whether TACI could

35   serve as a receptor for other members of the TNF family of ligands.

COS 7 cells (ATCC) were transiently transfected (using Lipofectamine

reagent) with membrane forms of various ligands of TNF family. Among

the ligands tested were APRIL, TALL-1, 4-1 BBL, CD27L, CD30L, CD40L,

EDA, FasL, GITRL, LT-alpha, OX-40L, RANKL, TNF-alpha, TNF-beta and

40   Apo2L/TRAIL.

Human TNF-alpha was cloned into pRK5B vector (pRK5B is a precursor of pRK5D that does not contain the SfiI site; see Holmes et al., Science, 253:1278-1280 (1991)). For the detection of TNF-alpha expression on the cell surface, a Flag tag was inserted between amino

5   acid 70 and amino acid 71 (using the numbering according to the sequence in Pennica et al., supra). An extracellular region of TALL-1 (aa 75-285; see Figure 3), 4-1BBL (aa 59-254; Goodwin et al., Eur. J. Immunol., 23:2631-2641 (1993)), CD27 ligand (aa 40-193; Goodwin et al., Cell, 73:447-456 (1993)), CD30 ligand (aa 61-234; Smith et al.,

10   Cell, 73:1349-1360 (1993)), RANKL (aa 71-317; see WO98/28426), Apo-2 ligand (aa 40-281; see WO97/25428) or Apo-3L (aa 46-249; see WO99/19490) was individually cloned at the BamHI site. This resulted in a chimeric ligand with the intracellular and transmembrane regions from TNF-alpha and the extracellular region from the various ligands.

15   For APRIL (see Figure 4) and EDA (Srivastava et al., supra), full length cDNA clones without Flag tag were used.

Transfected COS 7 cells were subsequently incubated with TACI.ECD.hFC immunoadhesin (prepared as described above). Cells were incubated with the TACI ECD-IgG (or a TNFR1-IgG construct prepared as

20   described in Ashkenazi et al., Proc. Natl. Acad. Sci., 88:10535-10539 (1991)) at 1 µg/ml for 1 hour in PBS. Cells were subsequently washed three times with PBS and fixed with 4% paraformaldehyde in PBS. Cell staining was visualized by incubation with biotinylated goat anti-human antibody (Jackson Labs, at 1:200 dilution) followed by Cy3-

25   streptavidin (Jackson Labs, at 1:200 dilution).

To identify potential ligand(s) of BCMA, similar binding experiments, as described above for TACI, were performed. A BCMA.ECD.hFc immunoadhesin was prepared, as described above.

Similar to TACI, BCMA only interacted with APRIL and TALL-1. As

30   shown in Fig. 9A, TACI-hFc and BCMA-hFc bound to cells transfected with TALL-1 or April but not TNF-alpha. Conversely, AP-TALL-1 or AP-APRIL specifically bound to cells transfected with TACI or BCMA (Fig. 9B).·

35   **EXAMPLE 3**

Induction of IgM production by TALL-1 and APRIL and Inhibition of the Induction by TACI-IgG and BCMA-IgG

Human peripheral blood mononuclear cells (PBMC) were isolated on

40   a Ficol gradient according to manufacturer's instructions (LSM media,

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ICN/Cappel, OH). Peripheral blood leukocytes (PBL) were then
obtained from the PBMC using standard removal of plastic-adherent
cells. PBLs were plated in 48-well dishes (3 x 10^4 cells/well on 0.3
ml of RPMI1640 medium containing 10% FBS) and incubated for 72 hours

5   at 37° C, 5% CO_2, with PBS (control), or IL-4 (100ng/ml, control, R &
D Systems, Minneapolis, MN), or Flag-TALL-1 (as described in Example
2 above) (1 μg/ml). For inhibition analysis, the cells were
incubated with each of the above in combination with 20 μg/ml of
TACI-IgG or BCMA-IgG (prepared as described in Example 2 above), or

10   an isotype-matched immunoadhesin control. Cell supernatants were
collected and analyzed for IgM levels using an IgM ELISA kit
according to manufacturer's instructions (Bethyl Laboratories, TX).

The results are shown in Figure 10. IL-4, used as a positive
control, induced IgM production compared to the control PBS. TALL-1

15   or APRIL induced at least as much IgM production as IL-4. The
combination of TALL-1 and APRIL showed no further induction of IgM
compared to each ligand alone. TACI-IgG did not block the effect of
IL-4, but it blocked the effect of TALL-1 and/or APRIL completely.

BCMA-IgG did not block the effect of IL-4, but blocked the

20   effect of TALL-1 and/or APRIL substantially, though not completely.
The control immunoadhesin did not block any of the ligands. These
results show that TALL-1 and APRIL can induce IgM production in PBL.
Moreover, the data show that TACI-IgG and BCMA-IgG can block the
effects of TALL-1 or APRIL on IgM production, confirming their

25   respective ability to bind to each ligand and demonstrating their
respective ability to block the ligand's activity on target cells.

EXAMPLE 4

Interaction between TALL-1 or APRIL with
30   #### TACI and/or BCMA Results in Activation of NF-kB

293 cells (ATCC) were seeded 24 hours before transfection at 1 x
10^5 cells/well into 12-well plates and transfected with 0.25 μg of
ELAM-luciferase reporter gene plasmid, 25 ng pRL-TK (Promega) and the

35   indicated amounts of each expression construct (see Figure 11).
Total amount of transfected DNA was kept constant at 1 mg by
supplementation with empty pRK5B vector (see Example 2). In some
assay wells, Flag-tagged ligands (prepared as described in Example 2)
were added at concentrations indicated 4 hours after transfection.

40   In other assay wells, the cells were co-transfected with full length

TALL-1 (Fig. 3) or RANKL (WO98/28426). Cells were harvested 20-24 hours after transfection and reporter gene activity determined with the Dual-Luciferase Reporter Assay System (Promega).

Only minimal activation of NF-kB was observed when TACI or BCMA

5   was expressed alone at low levels (such as at 0.1 ng). The activation of NF-kB, however, was greatly augmented by either addition of Flag-TALL-1 or Flag-APRIL (Fig. 11A), or by co-transfection with full length TALL-1 or APRIL (Fig. 11B; 11C).

Treatment of untransfected IM-9 cells with Flag-TALL-1 also

10   resulted in activation of NF-kB (see Fig. 11D). The IM-9 cells (ATCC) were incubated with Flag-TALL-1 (0.3 µg/ml) or PBS alone or in combination with 20 µg/ml TACI-IgG or TNFR1-IgG (prepared as described in Example 2). The NF-kB activity was measured by an electrophoretic mobility shift assay as described in Montgomery et

15   al., Cell, 87:427-436 (1996); Marsters et al., J. Biol. Chem., 272:14029 (1997); Chinnaiyan et al., Science, 274:990 (1996); Marsters et al., Curr. Biol., 6:1669 (1996); Pan et al., FEBS Letters, 431:351 (1998).

The data suggests that one physiological consequence of TALL-

20   1/APRIL-TACI/BCMA interaction is the activation of the NF-kB pathway.

EXAMPLE 5

Inhibition of Germinal Center Formation and Antibody Production in TACI-IgG or BCMA-IgG Treated, Immunized Mice

25

In vivo assays were conducted to determine whether the blocking of. the TALL-1/TACI or TALL-1/BCMA interaction impairs humoral immune responses. Three groups of female C57BL/6 mice of 6 - 8 week of age were immunized intraperitoneally (i.p.) with 100 µg of acetyl-

30   conjugated chicken gamma globulin (NP$_{23}$-CgG) (Biosource Technologies) precipitated in alum (4-hydroxy-3-nitrophenyl). The groups of animals were treated daily for 14 days with 50 µg of TACI-Fc or BCMA-Fc (prepared as described in Example 2) in 100 µl saline (and control animals were treated with 100 µl saline) by i.p. injection.

35   After 14 days, mouse sera were analyzed for NP-specific IgM, low-affinity IgG1, and high-affinity IgG1 using a standard ELISA method. NP-specific IgG1, both high-affinity antibodies and total (high- plus low-affinity) antibodies were quantified by ELISA in wells coated with NP$_{2.5}$ and NP$_{23}$-conjugated bovine serum albumin,

respectively. Bound antibodies were detected with AP-conjugated goat
anti-mouse IgM or IgG1 (Pharmingen).

The results are shown in Figure 12. TACI-Fc and BCMA-Fc
substantially inhibited the production of NP-specific IgM antibodies
5   as compared to control (Fig. 12A), indicating that TALL-1/TACI and
TALL-1/BCMA interactions (and APRIL/TACI and APRIL/BCMA interactions)
are important during the early phase of B cell activation that leads
to IgM secretion. TACI-Fc and BCMA-Fc inhibited low- and high-
affinity NP-specific IgG1 responses as well (Fig. 12 B, C),
10   suggesting that both TALL-1/TACI and TALL-1/BCMA interactions (and
both APRIL/TACI and APRIL/BCMA interactions) are important also for
Ig class switching and affinity maturation.

During the early part of an antigen-specific antibody response,
B cells differentiate into antibody-forming cells (AFC). This takes
15   place in extrafollicular areas of the spleen composed of
periarteriolar lymphoid sheaths (PALS) [Gray et al., Immunology,
65:73 (1988); NacLennan, Ann. Rev. Immunol., 12:117 (1988)], where Ig
class switching subsequently occurs. The PALS-associated regions
were compared from spleens of NP$_{23}$-CgG-immunized mice treated for 10
20   days with control Ig, TACI-Fc, or BCMA-Fc, similar to as described
above. Immunohistochemical analysis of the various spleen sections
was then conducted. Spleen sections prepared 10 days after
immunization and stained with FITC-conjugated anti-IgG1 are shown in
Figure 13-1 (Panel A) and Figure 13-2 (Panel A). As expected,
25   control mice displayed a large number of clustered AFC foci that
stained intensely with anti-IgG1 and contained many immunoblast-like
cells (Fig. 13-1, Panel A, left). In contrast, TACI-Fc treated mice
showed only few, isolated, IgG1-positive cells, with no formation of
AFC foci (Fig. 13-1, Panel A, right). BCMA-Fc treated mice likewise
30   showed only few, isolated, IgG1-positive cells, with no formation of
AFC foci (Fig. 13-2, Panel A). Thus, TALL-1/TACI and TALL-1/BCMA
(and APRIL/TACI and APRIL/BCMA) interactions are important for the
extrafollicular differentiation of B cells that precedes Ig class
switching in splenic PALS-associated areas.

35   To study the potential role of TALL-1/TACI and TALL-1/BCMA
interactions in antibody affinity maturation, the formation of
germinal centers (GC) was examined in the spleens of NP$_{23}$-CgG-
immunized mice at day 14. Spleen sections were prepared 14 days
after immunization and stained with FITC-conjugated anti-PNA (green
40   fluorescence) and Texas Red-conjugaed anti-IgM (red fluorescence).

90

As expected, splenic follicles from controls displayed intense
staining with peanut agglutinin (PNA), a lectin that binds
specifically to GC B cells (Fig. 13-1, Panel B, left). In sharp
contrast, splenic follicles from TACI-Fc treated mice were devoid of
5   GCs, and displayed only few, isolated, PNA-staining cells (Fig. 13-1,
Panel B, right). Splenic follicles from the BCMA-Fc treated mice
were also devoid of GCs, and displayed only few, isolated, PNA-
staining cells (Fig. 13-2, Panel B).

Despite the lack of GCs, there were no abnormalities in splenic
10   follicular architectures of TACI-Fc or BCMA-Fc treated mice, as
judged by hematoxilyn and eosin staining of spleen sections at day 14
(Figure 13-1 – Panel C, left (Controls) and Panel C, right (TACI-Fc
treated); and Figure 13-1 Panel C (BCMA-Fc treated). This suggests
that in TACI-Fc or BCMA-Fc treated mice, some follicular B cells
15   could differentiate into AFCs, but could not proceed to form GCs.
Thus, TALL-1/TACI and TALL-1/BCMA interactions (as well as APRIL/TACI
and APRIL/BCMA interactions) appear to be critical for proper GC
formation.

The blocking of the TALL-1/TACI and TALL-1/BCMA interactions (or
20   APRIL/TACI or APRIL/BCMA interactions) in mice during primary
immunization inhibited several aspects of the B cell response: (a)
the early phase of extrafollicular B cell activation that leads to
antigen-specific IgM production; (b) the differentiation of B cells
that leads to Ig class switching; (c) the formation of splenic GCs,
25   where affinity maturation occurs and memory B cells are generated.
While GC formation was blocked completely by TACI-Fc or BCMA-Fc, some
residual IgM and IgG1 production and affinity maturation occurred.
That attenuated antibody responses can proceed despite the absence of
GCs has been observed in other systems [see, e.g., Matsumoto et al.,
30   Nature, 382:462 (1996); Kato et al., J. Immunol., 160:4788 (1998);
Futtere et al., Immunity, 9:59 (1998). It is possible that other
factors besides TALL-1 or APRIL and TACI or BCMA mediate the
remaining antibody production. Alternatively, the selected TACI-Fc
or BCMA-Fc treatment in vivo may not have sufficed to prevent all
35   TALL-1/TACI, TALL-1/BCMA, APRIL/TACI or APRIL/BCMA binding events.

Previous studies indicate that CD40L-CD40 [Foy et al., Ann. Rev.
Immunol., 14:591 (1996)] and CD86-CD28/CTLA-4 [Han et al., J.
Immunol., 155:556 (1995); Lenschow et al., Ann. Rev. Immunol., 14:233
(1996)] interactions are important for entry of extrafollicular B
40   cells into GC areas and for GC establishment. Inhibition of these

interactions through gene knockouts or by treatment with blocking
antibodies or receptor-Fc fusions diminishes antibody production and
blocks GC formation [Lane et al., J. Exp. Med., 179:819 (1994); Durie
et al., Immunol. Today, 15:406 (1994); Hathcock et al., Science,
5   262:905 (1993); Linsey et al., Science, 257:7992 (1992); Renshaw et
al., J. Exp. Med., 180:1889 (1994); Xu et al., Immunity, 1:423
(1994); Kawabe et al., Immunity, 1:167 (1994); Foy et al., J. Exp.
Med., 180:157 (1994)]. There are some striking similarities between
the TALL-1/TACI, TALL-1/BCMA and CD40L-CD40 systems: both ligands are
10   related to TNF and are expressed on activated T cells and both
receptors are TNFR homologs that stimulate NF-KB and are expressed on
B cells. Hence, the interaction of TALL-1 or APRIL with TACI or BCMA
might mediate T-cell help to B cells similar to CD40L and CD40.
TALL-1 also may contribute to the activation of B cells by dendritic
15   cells, which do express the TALL-1 ligand. Unlike CD40L and CD40
knockout mice, which exhibit impaired IgG but not IgM responses, and
unlike CD40L-deficient patients with hyper-IgM syndrome [Callard et
al., Immunol. Today, 14:559 (1993); Allen at al., Science, 259:990
(1993); Aruffo et al., Cell, 72:291 (1993)], TACI-Fc-treated or BCMA-
20   Fc-treated mice showed a marked deficit in both IgM and IgG
production. Thus, it is possible that TALL-1 or APRIL and TACI or
BCMA operate early in B cell activation, such that their blockade
impairs all phases of the humoral response. In contrast, CD40L and
CD40 may operate later in B cell activation, such that their blockade
25   impairs only late phases of the antibody response.

EXAMPLE 6

Preparation of Anti-APRIL Monoclonal Antibodies

Balb/c mice (obtained from Charles River Laboratories) were
30   immunized by injecting 1.0µg of Flag-APRIL (diluted in MPL-TDM adjuvant
purchased from Ribi Immunochemical Research Inc., Hamilton, MT) 10
times into each hind foot pad. The immunization consisted of a series
of 6 injections (one injection/week for 6 weeks). The animals then
rested for two months, and subsequent immunization injections were
35   given once per week for 4 weeks. The Flag-tagged APRIL fusion protein
was prepared as described in Example 2 above and purified by anti-Flag
M2 agarose affinity chromatography (Sigma).

Three days after the final boost, popliteal lymph nodes were
removed from the mice and a single cell suspension was prepared in DMEM

media (obtained from Biowhitakker Corp.) supplemented with 1% penicillin-streptomycin. The lymph node cells were then fused with murine myeloma cells P3X63AgU.1 (ATCC CRL 1597) using 35% polyethylene glycol and cultured in 96-well culture plates. Hybridomas resulting

5   from the fusion were selected in HAT medium. Ten days after the fusion, hybridoma culture supernatants were screened in an ELISA to test for the presence of monoclonal antibodies binding to the Flag-APRIL protein. As a negative to discard monoclonal antibodies binding to the Flag portion or the molecule, the monoclonal antibodies were

10   also screened for any binding to Flag-tagged Apo-3.

In the ELISA, 96-well microtiter plates (Maxisorb; Nunc, Kamstrup, Denmark) were coated by adding 50 µl of .25 µg/ml Flag-APRIL or Flag-Apo-3 in 50mM carbonate buffer, pH 9.6, to each well and incubating at 4°C overnight. The plates were then washed three times

15   with wash buffer (PBS containing 0.05% Tween 20). The wells in the microtiter plates were then blocked with 200 µl of 2.0% bovine serum albumin in PBS and incubated at room temperature for 1 hour. The plates were then washed again three times with wash buffer.

Following the wash steps, 100 µl of the hybridoma supernatants or

20   various concentrations of polyclonal sera was added to designated wells. 100 µl of P3X63AgU.1 myeloma cell conditioned medium was added to other designated wells as controls. The plates were incubated at room temperature for 1 hour on a shaker apparatus and then washed three times with wash buffer.

25   Next, 50 µl HRP-conjugated goat anti-mouse IgG Fc (purchased from Cappel Laboratories), diluted 1:1000 in assay buffer (0.5% bovine serum albumin, 0.05% Tween-20, 0.01% Thimersol in PBS), was added to each well and the plates incubated for 1 hour at room temperature on a shaker apparatus. The plates were washed three times with wash buffer,

30   followed by addition of 50 µl of substrate (TMB microwell peroxidase substrate, Kirkegaard & Perry, Gaithersburg, MD) to each well and incubation at room temperature for 10 minutes. The reaction was stopped by adding 50 µl of TMB 1-component stop solution (diethyl glycol, Kirkegaard & Perry) to each well, and absorbance at 490 nm was

35   read in an automated microtiter plate reader.

The supernatants testing positive in the ELISA were then cloned twice by limiting dilution.

As shown in Figure 14A, the 3C6.4.2, 5E8.7.4, 5E11.1.2 and 5G8.2.2 antibodies were found to bind Flag-APRIL.

EXAMPLE 7

5   ### Isotyping of anti-APRIL Antibodies

The isotypes of the anti-APRIL monoclonal antibodies (see Example 6) were determined by coating plates with isotype specific goat anti-mouse Ig (Fisher Biotech, Pittsburgh, PA) at 4° C overnight. After non-specific binding sites were blocked with 2% BSA, 100 µl of

10   hybridoma culture supernatants or .5 µg/ml of purified mAbs were added. After incubation for 30 minutes at room temperature, plates were incubated with HRP-conjugated goat anti-mouse Ig for 30 minutes at room temperature. The level of HRP bound to the plate was detected using HRP substrate as described above.

15   As shown in the Table in Figure 14B, the anti-APRIL antibodies, 5E8.7.4, 5G8.2.2, and 3C6.4.2, were found to be isotype IgG2a antibodies. Anti-APRIL antibody 5E11.1.2 was found to be an isotype IgG1 antibody. ·

20   ## EXAMPLE 8

Binding Assay Showing Blocking Activity of anti-APRIL mAbs

Microtiter plates (Nunc, Denmark) were coated with 50 µl/well goat anti-human Fc antibody (Boehringer Manheim) at 5 µg/ml in carbonate buffer overnight at 4° C. The plates were then blocked with

25   150 µl/well of 2% BSA in PBS buffer for 1 hour at room temperature. The respective immunoadhesins, BCMA-IgG or TACI-IgG (prepared as described in Example 2 above) were added in a 50 µl/well volume at 5 µg/ml in block buffer and incubated at room temperature for 1 hour. All antibodies (which were identified in the fusion described in

30   Example 6) were diluted at 1:100 and 25 µl/well was added to the plate along with 25 µl/well of 2 µg/ml Flag-APRIL (see Example 2) and incubated for 1 hour at room temperature. The signal was developed with successive incubations with biotinylated anti-Flag antibody (Sigma Aldrich, Missouri) and streptavidin-horseradish peroxidase

35   (Amersham Life Science, New Jersey). All steps except the first were preceeded with a wash step with PBS/0.01% Tween 20.

Mab Identification	% Max Binding to BCMA	SD	% Max Binding to TACI	SD
3C6.4.2	10	0	18	0
5E8.7.4	96	2	88	6
5E11.1.2	65	0	52	4
5G8.2.2	101	5	94	9
IgG Ctrl	100	0	100	0

As shown in the Table above, anti-APRIL antibody 3C6.4.2 effectively blocked the APRIL binding to BCMA and to TACI. In the assay, antibody 5E11.1.2 also showed partial blocking of APRIL to BCMA
5   and to TACI.

EXAMPLE 9

Competitive Binding ELISA

To determine whether the anti-APRIL antibodies, 3C6.4.2,
10   5E8.7.4, 5E11.1.2 and 5G8.2.2 (described in the Examples above) recognized the same or different epitopes, a competitive binding ELISA was performed as described in J. Immunol. Methods, 156:9-17 (1992) using biotinylated anti-APRIL antibodies. The anti-APRIL monoclonal antibodies were biotinylated using N-hydroxyl succinimide
15   as described in J. Immunol. Methods, 156:9-17 (1992). Microtiter wells were coated with 50μl of 0.5μg/ml of Flag-APRIL (Example 2) in 50mM carbonate buffer, pH 9.6, overnight at 4° C. After washing, the nonspecific binding sites were blocked with 200μl of 2% BSA for 1 hour. After washing, a mixture of a predetermined optimal concentration of
20   biotinylated anti-APRIL antibodies and a 100-fold excess of unlabeled monoclonal antibodies were added to each well. Following a 1 hour incubation at room temperature, plates were washed and the amount of biotinylated anti-APRIL antibody was detected by the addition of HRP-streptavidin. After washing the microtiter wells, the bound enzyme was
25   detected by the addition of substrate, and the plates were read at 450nM with an ELISA plate reader.

The results are shown in Figure 15. The data shows that antibody 3C6.4.2 and antibody 5E11.1.2 may recognize shared epitopes since unlabeled antibodies 3C6.4.2 or 5E11.1.2 were able to inhibit the
30   binding of both biotinylated forms of the antibodies (Bio-3C6.4.2, Bio-5E11.1.2). Both antibodies 3C6.4.2 and 5E11.1.2 recognize different epitopes from those recognized by 5E8.7.4 and 5G8.2.2 since neither

antibodies blocked the binding of Bio-5E8.7.4 and Bio-5G8.2.2.
Further, antibodies 5E8.7.4 and 5G8.2.2 were able to block only its own
biotinylated monoclonal antibody but not others. Accordingly, among
the four monoclonal antibodies tested, it appears that three major
5   epitopes on APRIL were detected.

EXAMPLE 10

Effects of TACI Immunoadhesin in Murine Arthritis Model

An *in vivo* assay in a collagen-induced arthritis (CIA) murine
10   model was conducted to determine if inhibition of TACI interaction
with its ligand(s) could prevent progression of CIA.

Rheumatoid arthritis (RA) is an autoimmune disease in which the
synovial membrane of multiple joints can become inflamed, leading to
destruction of joint tissues including bone and cartilage. The
15   synovium of RA can be highly inflammatory in nature and is typically
characterized by lymphocyte and mononuclear cell infiltration, T cell
and antigen pressing cell (APC) activation, B cell immunoglobulin
(Ig) secretion and pro-inflammatory cytokine production [Potocnik et
al., Scand. J. Immunol., 31:213 (1990); Wernick et al., Arthritis
20   Rheum., 28:742 (1985); Ridley et al., Br. J. Rheumatology, 29:84
(1990); Thomas et al., J. Immunol., 152:2613 (1994); Thomas et al.,
J. Immunol., 156:3074 (1996)]. Chronically inflamed synovium is
usually accompanied by a massive CD4 T cell infiltration [Pitzalis et
al., Eur. J. Immunol., 18:1397 (1988); Morimoto et al., Am. J. Med.,
25   84:817 (1988)].

Collagen-induced arthritis (CIA) is an animal model for human
RA, which resembles human disease, and can be induced in susceptible
strains of mice by immunization with heterologous type-II collagen
(CII) [Courtenay et al., Nature, 283:665 (1980); Cathcart et al.,
30   Lab. Invest., 54:26 (1986)]. Both CD4 T cells and antibodies to CII
are required to develop CIA. Transfer of anti-CII to naïve animals
only leads to partial histo-pathology that is quite different from
CIA, and complete symptoms of CIA do not develop [Holmdahl et al.,
Agents Action, 19:295 (1986)]. In contrast, adoptive transfer of
35   both CD4 T cells and anti-CII antibodies from CII immunized mice to
naïve recipients completely reconstitutes the symptoms of classical
CIA [Seki et al., J. Immunol., 148:3093 (1992)]. Involvement of both
T cells and antibodies in CIA is also consistent with histo-

pathological findings of inflamed joints in CIA. Thus, agents that block B cell or T cell functions, or inhibit pro-inflammatory cytokines induced by T cells, may be efficacious to prevent or treat arthritis. Indeed, depletion of CD4 T cells, blockade of CD40-CD40L interactions, neutralization of TNF-alpha or blocking of IL-1 receptors can all lead to prevention of CIA in mice [Maini et al., Immunol. Rev., 144:195 (1995); Joosten et al., Arthritis Rheum., 39:797 (1996); Durie et al., Science, 261:1328 (1993)].

In Applicants' study, two groups of mice (7 to 8 week old male DBA/1 mice (Jackson Laboratory)) were immunized intradermally with 100 µg bovine collagen type-II (BCII) (Sigma Chemical Co.) emulsified in complete Freund's adjuvant (CFA) (Difco). The mice were then rechallenged with BCII in incomplete Freund's adjuvant 21 days later. A dramatic disease with clinical signs of arthritis developed in the animals that progressed to a more severe form with time. Starting on day 24, one group of mice were injected with 100 µg of TACI-Fc three times per week intraperitoneally for six weeks (N=9), and a second group received 100 µg of murine IgG as a control (N=10). The TACI-Fc construct was prepared by using primers based on the human TACI sequence (described herein) to amplify the mouse TACI cDNA from a mouse spleen library. A PCR product of about 0.45 kb was cloned. A cDNA clone containing the complete open reading frame of mouse TACI was subsequently isolated from the same library (GenBank accession number AF257673). The murine TACI-Fc was constructed by cloning the extracellular domain of mouse TACI (amino acids 2-129) between a pro-trypsin signal sequence and mouse IgG1-Fc sequence and the immunoadhesion prepared as described in the Examples above. Animals were then monitored for the clinical signs of arthritis, and at the end of the study, as described below, a radiological and histo-pathological examination was performed.

Mice were examined daily for signs of joint inflammation and scored as follows: 0, normal; 1, erythema and mild swelling confined to the ankle joint; 2, erythema and mild swelling extending from the ankle to metatarsal/metacarpal joints; 3, erythema and moderate swelling extending from the ankle to the metatarsophalangeal/metacarpophalengeal joints; 4, erythema and severe swelling extending from the ankle to the digits. The maximal arthritic score per foot is 4 and the maximal disease score per mouse is 16; the mean arthritic score was calculated from all animals in the group.

For radiological analysis at the end of the study, both fore-
and hind-paws were radio-graphed using X-ray Faxitron Imaging System
(Faxitron X-ray Corp., Wheeling, IL). Data was digitized and
pictures of radiographs were prepared. The radiographs were then
5   examined for bone erosion and soft tissue swelling. For histo-
pathological analysis, paws from the mice were excised, fixed in 10%
formalin, decalcified, and embedded in paraffin. Joint sections (6 –
8 μm) were prepared and stained with hematoxylin and eosin using
standard histochemical methods. Microscopic evaluation of arthritic
10 paws was performed in a blinded fashion. Arthritic changes in the
ankle,   metacarpophalangeal/metatarsophalangeal,   proximal
interphalangeal, and joints were examined for articular cartilage and
subchondral bone erosion.

Fig. 16A illustrates the disease courses in TACI-Fc treated mice
15 (circles), or control IgG treated mice mice (boxes) and saline
treated mice (triangles). Each data point represents a mean ± SD
from a total of 9 (for TACI-Fc treated group) or 10 (for control
groups) mice. The differences between the TACI-Fc treated group and
each of the two other groups are statistically significant. Mice in
20 the control group developed typical clinical symptoms of arthritis,
which started at about day 30 and progressed to very high arthritic
scores rapidly (Fig. 16A). In contrast, in the mice treated with
TACI-Fc, progression of arthritis was markedly inhibited. Fig. 16B
shows the disease scores of individual feet 3 weeks after the second
25 immunization. Each data point represents an individual foot. The
differences between the control and TACI-Fc-treated groups are
statistically significant. The arthritic scores in the TACI-Fc
treated mice reached only to 1.0, and that happened only towards the
end of study, whereas in the control group, the arthritic scores
30 reached to >7.0. These data clearly demonstrate that TACI
interaction with its ligand(s) is important for the development of
CIA.

To determine if the TACI-Fc treatment of the mice had any
effects on histo-pathology of the joints, at the end of the study
35 histo-pathological examination of the paws of mice was performed. At
the termination of the study (48 days after the second immunization),
the mice were sacrificed and their ankle joints were analyzed (HE
staining) for histology.

In   the   control   group,   a   severe   arthritic   disease   was
40 characterized   with   synovial   proliferation,   massive   leukocyte

98

infiltration, pinnus formation that resulted in articular cartilage
and bone erosion as shown in the proximal interphalangeal joint (Fig.
17A). Fig. 17A shows a phalangeal joint of a control mouse
indicating severe synovitis, hyperplasia, and cartilage and bone
5   destruction. Synovial thickening, leukocyte infiltration, articular
cartilage degeneration and periarticular erosion was also seen in the
metacarpal joint (Fig. 17B). Fig. 17B shows a phalangeal joint of a
TACI-Fc-treated mouse with no signs of synovitis or disease
pathology. In the TACI-Fc treated mice, there was no evidence of
10 histo-pathological symptoms, indicating that TACI-Fc not only blocks
clinical symptoms of CIA but also inhibits histo-pathological
symptoms (Fig. 17C,D). Fig. 17C shows a metacarpal of a control
mouse having disease pathology with massive signs of synovitis, and
Fig. 17D shows a metacarpal joint of a TACI-Fc-treated mouse with no
15 disease pathology.

At the termination of the study, both fore- and hind- paws of
the animals were also X-rayed and analyzed for bone structures as
described above. In the control group, signs of massive bone
destruction and disfiguration was apparent, whereas in the TACI-Fc
20 treated mice, no significant signs of bone loss or disfiguration was
seen (Fig. 17E,F). Fig. 17E shows a radiograph from a control mouse
showing signs of massive bone destruction and disfiguration. Fig.
17F shows a radiograph from a TACI-Fc treated mouse showing no
significant signs of bone loss or disfiguration. When radiographs
25 from the TACI-Fc treated mice were compared with those from naïve
mice; no apparent differences were revealed, indicating TACI-Fc
treatment completely protected mice from bone and cartilage damage
(Data not shown).

Since anti-collagen antibodies are believed to play an important
30 role in the development of arthritis, serum samples of the mice were
also analyzed to determine whether TACI-Fc treatment of the mice
resulted in inhibition of an anti-collagen humoral immune response.
To test humoral immune responses, the mice were bled retroorbitally
14 days (Fig. 18A) and 47 days (Fig. 18B) after the second
35 immunization and analyzed for the presence of anti-collagen
antibodies.

Serum levels of anti-BCII IgG1 and IgG2a isotypes were measured
by an ELISA using BCII collagen as antigen. In brief, microtiter
plates were coated with 10 µg/ml native bovine CII, blocked, and
40 incubated with serially diluted test sera. Bound IgG was detected by

incubation with alkaline phosphatase-conjugated goat anti-mouse IgG (Pharmingen), followed by substrate (dinitrophenyl phosphate). Optical densities were measured at 450 nm in an ELISA plate reader (Molecular Devices).

The results are shown in Fig. 18. Each data point represents a mean ± SD from five mice in each group. Serum from the mice in the control group showed presence of high levels of anti-collagen IgG1 and IgG2a, whereas in the TACI-Fc treated group, a considerable inhibition of both anti-collagen IgG1 and Ig2a was seen on days 14 and 47 after the second immunization. (Fig. 18A,B). Fig. 18A shows anticollagen IgG1 and IgG2a levels 14 days after the second immunization, and Fig. 18B shows anticollagen IgG1 and IgG2a levels 47 days after the second immunization (White bars, mice treated with BSA; black bars, mice treated with TACI-Fc). These results suggest that TACI-Fc treatment may attune the development of CIA, at least partially, by blocking anti-collagen antibodies.

Since both collagen-specific B and T cells can initiate CIA, an assay was further conducted to examine whether prevention of TACI-Fc mediated CIA was also associated with inhibition of T cell effector functions. Lymph nodes and spleens from both the control and TACI-Fc treated mice were collected at the end of the study and in vitro recall responses of T cells against collagen and production of effector cytokines was examined. BCII immunized mice were sacrificed 47 days after the second immunization, and their inguinal lymph nodes and spleen were collected. Single cell suspensions were prepared, and cells were cultured in 96-well plates at a density of 1×10^6 cells/ml (200 µl/well) in DMEM containing 5% heat-inactivated FCS, 2 mM glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, and 2×10^{-5} M 2-ME. Cells were cultured in medium alone, or in the presence of various concentrations BCII. To test lymphocyte proliferation (Fig. 18C), lymph node cells (1×10^6 cells per well) were first cultured for 72 hours, followed by addition of 1 µCi of [^{3}H] thymidine (International Chemical and Nuclear, Irvine, CA) for the last 18 hours of a 5-day culture, and incorporation of radioactivity was assayed by liquid scintillation counting (represented as cpm) using a Wallac ß-plate counter.

Proliferative T cell responses against collagen from TACI-Fc treated mice were almost negligible as compared to that of control mice (Fig. 18C; control mice -boxes; TACI-Fc treated mice- circles).

For the cytokine assays, the lymph node and splenic cells were cultured in 0.2 ml of medium with or without BCII; 1×10^6 cells/ml (200 μl/well) were cultured in the above-mentioned medium alone, or in the presence BCII. Supernatants were collected after 24 hours to

5 test for for IL-2 secretion and 72 hours later to test for for IFN-gamma production, which was found to be the optimal incubation time for cytokine determination, and stored at -20°C until analyzed. Levels of IL-2 and IFN-gamma were detected by ELISA using a kit from Pharmingen (San Deigo, California). Standard curves were generated

10 using mouse recombinant IL-2 and IFN-gamma. When IL-2 and IFN-gamma production by T cells from these mice was measured, the TACI-Fc treated group (shown in Figs. 18D and 18E by circles) showed very little production of these cytokines, whereas T cells from the control group (shown in Figs. 18D and 18E by boxes) secreted

15 significant levels of both IL-2 and IFN-gamma (Fig. 18D (IL-2 production), 18E (IFN-gamma production)).

These data suggest that TACI-Fc treatment of mice not only inhibited anti-collagen antibody production but also regulated functions of effector T cells. Thus, TACI interactions with its

20 ligand(s) are also believed to be important in T cell mediated immune responses.

Since TACI receptor is also shown to be expressed on T cells and is involved in activation NF-AT associated with activation of T cells [von Bulow et al., Science, 278:138 (1997)], it is believed that

25 blocking of TACI interaction with its ligand(s) may directly impair T cell activation and its effector functions that are required, for instance, for the progression of CIA in mice.

To determine the direct role of TACI in T cell activation, an *in vitro* assay of antigen-specific activation of T cells was performed.

30 Activation of T cells by anti-CD3 antibody *in vitro* in the presence of TACI-Fc was examined by measuring proliferation and IL-2 production by these T cells. Splenic cells from adult C57BL/6 mice (Jackson Laboratory) were cultured (1×10^6 per well) in various concentrations of 10 μg/ml anti-CD3 monoclonal antibody (Pharmingen)

35 with or without different concentrations of TACI-Fc in medium as described above. Proliferation was measured by uptake of ^{3}H-thymidine as stated above. Parallel assays were also set up to measure the effects of TACI-Fc on production of anti-CD3 antibody induced IL-2 production in a 24 hour culture system as mentioned above. An ELISA

40 was used to determine IL-2 levels in supernatants, using antibodies

101

from Pharmingen, and using their recommended protocols. To study the effects of TACI-Fc on *in vitro* stimulation of TCR transgenic cells, 1 X 10^6 cells from adult MBP-TCR transgenic mice (bred from animal breeding pair obtained from Dr. Richard Flavell, Howard Hughes Medical Institute, Yale University) were cultured in the presence of 10 μg/ml MBP-Acl-11 (a synthetic NH2-terminal peptide of Myelin Basic Protein having amino acid sequence ASQKRPSQRSK (SEQ ID NO:10) with the first amino acid acetylated) with or without different concentrations of TACI-Fc in 96-well plates in DMEM medium supplemented with 5% FCS, 2 mM glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin. Proliferation was measured by addition of 1 μCi of [^{3}H] thymidine (International Chemical and Nuclear, Irvine, CA) for the last 18 hours of a 5-day culture, and incorporation of radioactivity was assayed by liquid scintillation counting.

Fig. 19A shows the inhibition of anti-CD3 antibody-induced proliferation of naive T cells by TACI-Fc in a dose dependent manner, while Fig. 19B shows the inhibition of anti-CD3 antibody-induced IL-2 production by naive T cells, as affected by TACI-Fc in a dose dependent manner. (In Figs. 19A and 19B, TACI-Fc treatment is shown by circles; controls are shown by boxes).

Activation of myelin basic protein (MBP)-TCR transgenic T cells by antigen *in vitro* in the presence of TACI-Fc were also examined by measuring proliferation and IL-2 production by these T cells (as described above). Again, TACI-Fc inhibited both proliferation and IL-2 production by MBP-TCR transgenic T cells in a dose dependent manner (data not shown). These results demonstrate that TACI receptor is involved in T cell activation, and that this function can be blocked with TACI-Fc.

EXAMPLE 11

Effects of TACI Immunoadhesin in Murine EAE Model

The EAE murine model been described in the literature as a model for human multiple sclerosis [Grewal et al., *Science*, 273:1864-1867 (1996).

In Applicants' study, two groups of 10 mice each (10 to 15 week old male and female MBP-TCR transgenic mice (described in Example 10) were immunized subcutaneously with 10 μg MBP Acl-11 (described in Example 10 above) in 100 μl complete Freund's adjuvant (CFA) (Difco). Following the initial immunization with Acl-11, 200 ng Pertussis

toxin (List Biologicals, Campbell, CA) in 100 µl saline was injected intraperitoneally in each mouse at 24 hours and 48 hours. Starting on day 2 through day 24, one group of mice was injected with 100 µg of TACI-Fc (described in Example 10) in 100 µl sterile saline

5   intraperitoneally daily, and a second group received 100 µg of murine IgG in 100 µl sterile saline intraperitoneally each day. Animals were then monitored daily for the onset of disease. Clinical signs of experimental allergic encephalomyelitis (EAE) were assessed daily and a score of 1 to 5 was given to each mouse based on the

10   established EAE index system: 0= normal appearance; 1= tail droop; 2= abnormal gait; 3= limb weakness; 4= paralysis involving one limb (partial hindlimb paralysis); 5= paralysis involving two limbs (total hindlimb paralysis). This is a modified scoring system from that previously described in Grewal et al., 273:1864-1867 (1996).

15   The results are shown in Figure 20. The data shown in Figure 20 indicate that animals receiving the control IgG developed expected clinical symptoms of EAE; the disease onset in the control treated mice started on day 5 and reached the peak levels within 10 days. In contrast, the TACI-Ig treated mice did not develop severe forms of

20   the EAE symptoms. The disease score was much lower than the control group, only reaching clinical scores of 2 (which did not progress to higher scores during the study). The results thus suggest that the TACI-Ig treatment protected the mice from developing overt EAE.

25   *********************************

Deposit of Material

          The following materials have been deposited with the
American Type Culture Collection, 10801 University Blvd., Manassas,
VA 20110-2209, USA (ATCC):

30   Material   ATCC Dep. No.   Deposit Date

     3C6.4.2   PTA-1347   Feb. 15, 2000
     5E11.1.2   PTA-1346   Feb. 15, 2000
     5G8.2.2   PTA-1345   Feb. 15, 2000
     5E8.7.4   PTA-1344   Feb. 15, 2000

35

          This deposit was made under the provisions of the Budapest
Treaty on the International Recognition of the Deposit of
Microorganisms for the Purpose of Patent Procedure and the
Regulations thereunder (Budapest Treaty). This assures maintenance

of a viable culture of the deposit for 30 years from the date of
deposit. The deposit will be made available by ATCC under the terms
of the Budapest Treaty, and subject to an agreement between
Genentech, Inc. and ATCC, which assures permanent and unrestricted

5   availability of the progeny of the culture of the deposit to the
public upon issuance of the pertinent U.S. patent or upon laying open
to the public of any U.S. or foreign patent application, whichever
comes first, and assures availability of the progeny to one
determined by the U.S. Commissioner of Patents and Trademarks to be

10   entitled thereto according to 35 USC '122 and the Commissioner's
rules pursuant thereto (including 37 CFR '1.14 with particular
reference to 886 OG 638).

     The assignee of the present application has agreed that if a
culture of the materials on deposit should die or be lost or

15   destroyed when cultivated under suitable conditions, the materials
will be promptly replaced on notification with another of the same.
Availability of the deposited material is not to be construed as a
license to practice the invention in contravention of the rights
granted under the authority of any government in accordance with its

20   patent laws.

     The foregoing written description is considered to be sufficient
to enable one skilled in the art to practice the invention. The
present invention is not to be limited in scope by the example
presented herein. Indeed, various modifications of the invention in

25   addition to those shown and described herein will become apparent to
those skilled in the art from the foregoing description and fall
within the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A method of inhibiting or neutralizing TALL-1 polypeptide biological activity in mammalian cells, comprising exposing said mammalian cells to an effective amount of TALL-1 polypeptide antagonist.

2. The method of claim 1 wherein said TALL-1 polypeptide comprises a native sequence TALL-1 polypeptide having the amino acid sequence of Figure 3 (SEQ ID NO:6) or a fragment thereof which exhibits a biological activity of the native sequence TALL-1 polypeptide shown in Figure 3 (SEQ ID NO:6).

3. The method of claim 1 wherein said TALL-1 polypeptide antagonist comprises a TACI receptor immunoadhesin.

4. The method of claim 3 wherein said TACI receptor immunoadhesin comprises a TACI extracellular domain sequence fused to an Fc region of an immunoglobulin.

5. The method of claim 1 wherein said TALL-1 polypeptide antagonist comprises a BCMA receptor immunoadhesin.

6. The method of claim 5 wherein said BCMA receptor immunoadhesin comprises a BCMA extracellular domain sequence fused to an Fc region of an immunoglobulin.

7. The method of claim 1 wherein said TALL-1 polypeptide antagonist comprises a TACI receptor variant.

8. The method of claim 1 wherein said TALL-1 polypeptide antagonist comprises a BCMA receptor variant.

9. The method of claim 1 wherein said TALL-1 polypeptide antagonist comprises a TACI receptor linked to a nonproteinaceous polymer selected from the group consisting of polyethylene glycol, polypropylene glycol, and polyoxyalkylene.

10. The method of claim 1 wherein said TALL-1 polypeptide antagonist comprises a BCMA receptor linked to a nonproteinaceous polymer selected from the group consisting of polyethylene glycol, polypropylene glycol, and polyoxyalkylene.

11. The method of claim 1 wherein said TALL-1 polypeptide antagonist comprises a TACI receptor polypeptide or BCMA receptor polypeptide linked to a heterologous sequence.

12. The method of claim 11 wherein said heterologous sequence is an epitope tag sequence.

13. The method of claim 11 wherein said heterologous sequence is a leucine zipper sequence.

14. The method of claim 1 wherein said TALL-1 polypeptide antagonist comprises an antibody selected from the group consisting of a TACI receptor antibody, a BCMA receptor antibody, a TALL-1 polypeptide antibody and an APRIL polypeptide antibody.

15. The method of claim 1 wherein said TALL-1 polypeptide antagonist comprises an extracellular domain sequence of TACI receptor or an extracellular domain sequence of BCMA receptor.

16. The method of claim 1 wherein said TALL-1 polypeptide antagonist comprises an antagonist molecule which inhibits or neutralizes both TALL-1 polypeptide and APRIL polypeptide biological activity in mammalian cells.

17. The method of claim 1 wherein said mammalian cells comprise white blood cells.

18. A method of inhibiting or neutralizing TALL-1 polypeptide biological activity in mammalian cells, comprising exposing said mammalian cells to an effective amount of an antagonist which inhibits or neutralizes a biological activity of a native sequence TALL-1 polypeptide having the amino acid sequence of Figure 3 (SEQ ID NO:6) or a fragment thereof.

19. A method of inhibiting or neutralizing APRIL polypeptide
biological activity in mammalian cells, comprising exposing said
mammalian cells to an effective amount of APRIL polypeptide
antagonist.

5

20. The method of claim 19 wherein said APRIL polypeptide
comprises a native sequence APRIL polypeptide having the amino acid
sequence of Figure 4 (SEQ ID NO:8) or a fragment thereof which
exhibits a biological activity of the native sequence APRIL

10   polypeptide shown in Figure 4 (SEQ ID NO:8).

21. The method of claim 19 wherein said APRIL polypeptide
antagonist comprises a TACI receptor immunoadhesin.

15   22. The method of claim 21 wherein said TACI receptor
immunoadhesin comprises a TACI extracellular domain sequence fused to
an Fc region of an immunoglobulin.

23. The method of claim 19 wherein said APRIL polypeptide
20   antagonist comprises a BCMA receptor immunoadhesin.

24. The method of claim 23 wherein said BCMA receptor
immunoadhesin comprises a BCMA extracellular domain sequence fused to
an Fc region of an immunoglobulin.

25

25. The method of claim 19 wherein said APRIL polypeptide
antagonist comprises a TACI receptor variant.

26. The method of claim 19 wherein said APRIL polypeptide
30   antagonist comprises a BCMA receptor variant.

27. The method of claim 19 wherein said APRIL polypeptide
antagonist comprises a TACI receptor linked to a nonproteinaceous
polymer selected from the group consisting of polyethylene glycol,
35   polypropylene glycol, and polyoxyalkylene.

28. The method of claim 19 wherein said APRIL polypeptide
antagonist comprises a BCMA receptor linked to a nonproteinaceous
polymer selected from the group consisting of polyethylene glycol,
40   polypropylene glycol, and polyoxyalkylene.

29. The method of claim 19 wherein said APRIL polypeptide antagonist comprises a TACI receptor polypeptide or BCMA receptor polypeptide linked to a heterologous sequence.

5   30. The method of claim 29 wherein said heterologous sequence is an epitope tag sequence.

31. The method of claim 29 wherein said heterologous sequence is a leucine zipper sequence.

10

32. The method of claim 19 wherein said APRIL polypeptide antagonist comprises an antibody selected from the group consisting of a TACI receptor antibody, a BCMA receptor antibody, a TALL-1 polypeptide antibody and an APRIL polypeptide antibody.

15

33. The method of claim 32 wherein said APRIL polypeptide antagonist comprises the anti-APRIL antibody 3C6.4.2 deposited as ATCC accession number PTA-1347.

20   34. The method of claim 19 wherein said APRIL polypeptide antagonist comprises an extracellular domain sequence of TACI receptor or an extracellular domain sequence of BCMA receptor.

35. The method of claim 19 wherein said APRIL polypeptide
25   antagonist comprises an antagonist molecule which inhibits or neutralizes both TALL-1 polypeptide and APRIL polypeptide biological activity in mammalian cells.

36. The method of claim 19 wherein said mammalian cells comprise
30°   white blood cells.

37. A method of inhibiting or neutralizing APRIL polypeptide biological activity in mammalian cells, comprising exposing said mammalian cells to an effective amount of an antagonist which
35   inhibits or neutralizes a biological activity of a native sequence APRIL polypeptide having the amino acid sequence of Figure 4 (SEQ ID NO:8) or a fragment thereof.

38. A method of enhancing or stimulating TACI polypeptide activity in mammalian cells, comprising exposing said mammalian cells to an effective amount of TACI polypeptide agonist.

5   39. The method of claim 38 wherein said TACI polypeptide agonist comprises an anti-TACI agonist antibody.

40. The method of claim 38 wherein said TACI polypeptide agonist mimics the activity of APRIL polypeptide or TALL-1 polypeptide.

10

41. A method of enhancing or stimulating BCMA polypeptide activity in mammalian cells, comprising exposing said mammalian cells to an effective amount of BCMA polypeptide agonist.

15   42. The method of claim 41 wherein said BCMA polypeptide agonist comprises an anti-BCMA agonist antibody.

43. The method of claim 41 wherein said BCMA polypeptide agonist mimics the activity of APRIL polypeptide or TALL-1 polypeptide.

20

44. A method of treating a TALL-1 -related pathological condition in a mammal, comprising administering to said mammal an effective amount of TALL-1 antagonist.

25   45. The method of claim 44 wherein said TALL-1 antagonist is selected from the group consisting of a soluble, extracellular domain sequence of TACI or BCMA, TACI receptor immunoadhesin, BCMA receptor immunoadhesin, TACI receptor variant, BCMA receptor variant, pegylated TACI receptor, pegylated BCMA receptor, TACI receptor
30 fusion protein, BCMA receptor fusion protein, TACI antibody, BCMA antibody, TALL-1 antibody, and APRIL antibody.

46. The method of claim 44 wherein said pathological condition is an immune related disease.

35

47. The method of claim 46 wherein said immune related disease is an autoimmune disease.

48. The method of claim 46 wherein said immune related disease
40 comprises rheumatoid arthritis.

49. The method of claim 44 wherein said pathological condition
is multiple sclerosis.

5   50. The method of claim 44 wherein said pathological condition
is cancer.

51. The method of claim 50 wherein said cancer is leukemia,
lymphoma, or myeloma.
10

52. The method of claim 50 wherein said cancer is colon, lung,
or melanoma cancer.

53. The method of claim 44 wherein, in addition to administering
15   said TALL-1 antagonist, one or more cytotoxic agents,
chemotherapeutic agents, cytokines, or growth inhibitory agents are
administered to said mammal.

54. A method of treating an APRIL-related pathological condition
20   in a mammal, comprising administering to said mammal an effective
amount of APRIL antagonist.

55. The method of claim 54 wherein said APRIL antagonist is
selected from the group consisting of a soluble, extracellular domain
25   sequence of TACI or BCMA, TACI receptor immunoadhesin, BCMA receptor
immunoadhesin, TACI receptor variant, BCMA receptor variant,
pegylated TACI receptor, pegylated BCMA receptor, TACI receptor
fusion protein, BCMA receptor fusion protein, TACI antibody, BCMA
antibody, TALL-1 antibody, and APRIL antibody.
30

56. The method of claim 54 wherein said pathological condition
is an immune related disease.

57. The method of claim 56 wherein said immune related disease
35   is an autoimmune disease.

58. The method of claim 56 wherein said immune related disease
comprises rheumatoid arthritis.

59. The method of claim 54 wherein said pathological condition is multiple sclerosis.

60. The method of claim 54 wherein said pathological condition is cancer.

61. The method of claim 60 wherein said cancer is leukemia, lymphoma, or myeloma.

62. The method of claim 54 wherein, in addition to administering said TALL-1 antagonist, one or more cytotoxic agents, chemotherapeutic agents, cytokines, or growth inhibitory agents are administered to said mammal.

63. A composition comprising a TALL-1 polypeptide antagonist and a carrier.

64. The composition of claim 63 wherein said carrier comprises a pharmaceutically acceptable carrier.

65. A composition comprising an APRIL antagonist and a carrier.

66. The composition of claim 65 wherein said carrier comprises a pharmaceutically acceptable carrier.

67. A monoclonal antibody which specifically binds to APRIL polypeptide and blocks binding of said APRIL polypeptide to a TACI receptor or a BCMA receptor.

68. The monoclonal antibody of claim 67 which further blocks binding of said APRIL polypeptide to a TACI receptor and a BCMA receptor.

69. The monoclonal antibody of claim 67 wherein said monoclonal antibody comprises the 3C6.4.2 antibody secreted by the hybridoma deposited with ATCC as accession number PTA-1347.

70. A monoclonal antibody which binds to the same epitope as the epitope to which the 3C6.4.2 monoclonal antibody produced by the hybridoma cell line deposited as ATCC accession number PTA-1347 binds.

71. The hybridoma cell line which produces monoclonal antibody 3C6.4.2 and deposited with ATCC as accession number PTA-1347.

72. The monoclonal antibody 5E11.1.2 secreted by the hybridoma deposited with ATCC as accession number PTA-1346.

73. A monoclonal antibody which binds to the same epitope as the epitope to which the 5E11.1.2 monoclonal antibody produced by the hybridoma cell line deposited as ATCC accession number PTA-1346 binds.

74. The hybridoma cell line which produces monoclonal antibody 5E11.1.2 and deposited with ATCC as accession number PTA-1346.

75. The monoclonal antibody 5G8.2.2 secreted by the hybridoma deposited with ATCC as accession number PTA-1345.

76. A monoclonal antibody which binds to the same epitope as the epitope to which the 5G8.2.2 monoclonal antibody produced by the hybridoma cell line deposited as ATCC accession number PTA-1345 binds.

77. The hybridoma cell line which produces monoclonal antibody 5G8.2.2 and deposited with ATCC as accession number PTA-1345.

78. The monoclonal antibody 5E8.7.4 secreted by the hybridoma deposited with ATCC as accession number PTA-1344.

79. A monoclonal antibody which binds to the same epitope as the epitope to which the 5E8.7.4 monoclonal antibody produced by the hybridoma cell line deposited as ATCC accession number PTA-1344 binds.

80. The hybridoma cell line which produces monoclonal antibody 5E8.7.4 and deposited with ATCC as accession number PTA-1344.

81. A chimeric anti-APRIL antibody which specifically binds to APRIL polypeptide and comprises a sequence derived from the 3C6.4.2 antibody secreted by the hybridoma deposited with ATCC as accession
5   number PTA-1347.

82. A chimeric anti-APRIL antibody which specifically binds to APRIL polypeptide and comprises a sequence derived from the 5E11.1.2 antibody secreted by the hybridoma deposited with ATCC as accession
10   number PTA-1346.

83. A chimeric anti-APRIL antibody which specifically binds to APRIL polypeptide and comprises a sequence derived from the 5G8.2.2 antibody secreted by the hybridoma deposited with ATCC as accession
15   number PTA-1345.

84. A chimeric anti-APRIL antibody which specifically binds to APRIL polypeptide and comprises a sequence derived from the 5E8.7.4 antibody secreted by the hybridoma deposited with ATCC as accession
20   number PTA-1344.

85. A humanized anti-APRIL antibody which specifically binds to APRIL polypeptide and comprises a sequence derived from the 3C6.4.2 antibody secreted by the hybridoma deposited with ATCC as accession
25   number PTA-1347.

86. A humanized anti-APRIL antibody which specifically binds to APRIL polypeptide and comprises a sequence derived from the 5E11.1.2 antibody secreted by the hybridoma deposited with ATCC as accession
30   number PTA-1346.

87. A humanized anti-APRIL antibody which specifically binds to APRIL polypeptide and comprises a sequence derived from the 5G8.2.2 antibody secreted by the hybridoma deposited with ATCC as accession
35   number PTA-1345.

88. A humanized anti-APRIL antibody which specifically binds to APRIL polypeptide and comprises a sequence derived from the 5E8.7.4 antibody secreted by the hybridoma deposited with ATCC as accession
40   number PTA-1344.

Fig. 1A

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  1  AGCATCCTGA GTAATGAGTG GCCTGGGCCG GAGCAGGCGA GGTGGCCGGA GCCGTGTGGA CCAGGAGGAG CGCTTTCCAC AGGGCCTGTG GACGGGGGTG
     TCGTAGGACT CATTACTCAC CGGACCCGGC CTCGTCCGCT CCACCGGCCT CGGCACACCT GGTCCTCCTC GCGAAAGGTG TCCCGGACAC CTGCCCCCAC
  1            M  S  G     L  G  R     S  R  R     G  G  R     S  R  V     D  Q  E     E  R  F  P  Q  G  L     W  T  G  V

101  GCTATGAGAT CCTGCCCCGA AGAGCAGTAC TGGGATCCTC TGCTGGGTAC TTTGCAACCA TGCAAAACCA TCTGCAATCC CGCACCTGTG CGCTGGACAC
     CGATACTCTA GGACGGGGCT TCTCGTCATG ACCCTAGGAG ACGACCCATG AAACGTTGGT ACGTTTTGGT AGACGTTAGG GCGTGGACAC GCGACCTGTG
 30  A  M  R  S  C  P  E     E  Q  Y     W  D  P     L  G  T     C  M  S     C  K  T  I  C  N  H  Q  S  Q  R  T  C  A

201  CAGCCTTCTG CAGGTCACTC AGTCGCCGCA AGGAGCAAGG CAAGTTCTAT GACCATCTCC TGAGGGACTG CATCAGCTGT GCCTCCATCT GTGGACAGCA
     GTCGGAAGAC GTCCAGTGAG TCAGCGGCGT TCCTCGTTCC GTTCAAGATA CTGGTAGAGG ACTCCCTGAC GTAGTCGACA CGGAGGTAGA CACCTGTCGT
 64  A  F  C  R  S  L     S  C  R  K     E  Q  G     K  F  Y     D  H  L  L     R  D  C     I  S  C     A  S  I  C  G  Q  H

301  CCCTAAGCAA TGTGCATACT TCTGTGAGAA CAAGCTCAGG AGCCCAGTGA ACCTTCCACC AGAGCTCAGG AGACAGCGGA GTGGAGAAGT TGAAAACAAT
     GGGATTCGTT ACACGTATGA AGACACTCTT GTTCGAGTCC TCGGGTCACT TGGAAGGTGG TCTCGAGTCC TCTGTCGCCT CACCTCTTCA ACTTTTGTTA
 97  P  K  Q  C  A  Y  F     C  E  N     K  L  R     S  P  V  N     L  P  P     E  L  R     Q  R  S     G  E  V  E  N  N

401  TCAGACAACT CGGGAGGTA CCAAGGATTG GAGCACAGAG GCTCAGAAGC AAGTCCAGCT CTCCCGGGGC TGAAGCTGAG TGCAGATCAG GTGGCCCTGG
     AGTCTGTTGA GCCCTCCAT GGTTCCTAAC CTCGTGTCTC CGAGTCTTCG TTCAGGTCGA GAGGGCCCCG ACTTCGACTC ACGTCTAGTC CACCGGGACC
130  S  D  N  S  G  R  Y     Q  G  L     E  H  R  G     S  E  A     S  P  A     L  P  G  L     K  L  S     A  D  Q  V  A  L  V

501  TCTACAGCAC GCTGGGGCTC TGCCTGTGTG CCGTCCTCTG CTGCTTCCTG GTGGCGGTG GCGTGCTTCC TGAAGAAGAG GGGGATCCCC CCCTGCAGCT
     AGATGTCGTG CGACCCCGAG ACGGACACAC GGCAGGAGAC GACGAAGGAC CACCGCCAC CGCACGAAGG ACTTCTTCTC CCCCTAGGGG GGGACGTCGA
164  Y  S  T  L  G  L  C     L  C  A     V  L  C     C  F  L     V  A  V  A     C  F  L     K  K  R     G  D  P  P  C  S  C  Q

601  GCCCCGCTCA AGGCCCCGTC AAAGTCCGGC CAAGTCTTCC CAGGATCACG CGATGGAAGC CGGCAGCCCT GTGAGCACAT CCCCCGAGCC AGTGGAGACC
     CGGGGCGAGT TCCGGGGCAG TTTCAGGCCG GTTCAGAAGG GTCCTAGTGC GCTACCTTCG GCCGTCGGGA CACTCGTGTA GGGGGCTCGG TCACCTCTGG
197  P  R  S  R  P  R  Q     S  P  A  K     S  S     Q  D  H  A     M  E  A     G  S  P     V  S  T  S     P  E  P  V  E  T

701  TGCAGCTTCT GCTTCCCTGA GTGCAGGGCG CCCACGCAGG AGAGCGCAGT CACGGCCTGG ACCCCCGACC CCACTTGTGC TGGAAGGTGG GGGTGCCACA
     ACGTCGAAGA CGAAGGGACT CACGTCCCGC GGGTGCGTCC TCTCGCGTCA GTGCCGGACC TGGGGGCTGG GGTGAACACG ACCTTCCACC CCCACGGTGT
230  C  S  F  C  F  P  E     C  R  A     P  T  Q  E     S  A  V     T  P  G     T  P  D  P     T  C  A     G  R  W  G  C  H  T

801  CCAGGACCAC AGTCCTGCAG CCTTGCCCAC ACATCCCAGA CAGTGGCCTT GGCATTGTGT GTGTGCCTGC CCAGGAGGGG GGCCCAGGTG CATAAATGGG
     GGTCCTGGTG TCAGGACGTC GGAACGGGTG TGTAGGGTCT GTCACCGGAA CCGTAACACA CACACGGACG GGTCCTCCCC CCGGGTCCAC GTATTTACCC
264  R  T  T  V  L  Q  P     C  P  H     I  P  D     S  G  L  G     I  V  C     V  P  A     Q  E  G  G     P  P  G  A  Q
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 901  GGTCAGGGAG GGAAAGGAGG AGGGAGAGAG ATGGAGAGGA GGGGAGAGGA AAAGAGAGGT GGGGAGAGGG GAGAGAGATA TGAGGAGAGA GAGACAGAGG
      CCAGTCCCTC CCTTTCCTCC TCCCTCTCTC TACCTCTCTC CCCCTCTCCC TTTCTCTCCA CCCCTCTCCC CTCTCTCTAT ACTCCTCTCT CTCTGTCTCC

1001  AGGCAGAAAC GGAGAGAAAC AGAGGAGACA GAGAGGGAGA GGGAGAGAGA GACAGAGGGG AAGAGAGGCA GAGAGGGAAA GAGGCAGAGA
      TCCGTCTTTG CCCTCTCTTC TCTCTCTCGT CTCTCTCTCT CCCTCTGTCT CTGTCTCCCC TTCTCTCCGT CTCTCCCTTT CTCCGTCTCT

1101  AGGAAAGAGA CAGGCAGAGA AGGAGAGAGG CAGAGAGGGA GAGGGAGAGA GAGGGAGAGA GGCAGAGAGA GAGAGGGACA
      TCCTTTCTCT GTCCGTCTCT TCCTCTCTCC GTCTCGTCCT CTCCCCTCT  CCGTCTCTCT CCGTCTCCCT GTCTCCCTGT CTCTCTCTAT

1201  GAGCAGGAGG TCGGGGCACT CTGAGTCCCA GTTCCCAGTG TCGTCATCAC CTAACCACAC GTGCAATAAA GTCCTCGTGC CTGCTGCTCA
      CTCGTCCTCC AGCCCCGTGA GACTCAGGGT CAAGGGTCAC AGCAGCATCC GATTGGTGTG CACGTTATTT CAGGAGCACG GACGACGAGT

1301  CAGCCCCCGA GAGCCCCTCC TCCTGGAGAA TAAAACCTTT GGCAGCTGCC CTTCCTCAAA AAAAAAAAAA AAAAAAA
      GTCGGGGGCT CTCGGGGAGG AGGACCTCTT ATTTTGGAAA CCGTCGACGG GAAGGAGTTT TTTTTTTTTT TTTTTTT
```

Fig. 1B

Fig. 2

```
  1  AAGACTCAAA CTTAGAAACT TGAATTAGAT GTGGTATTCA AATCCTTACG TGCCGCGAAG ACACAGACAG CCCCCGTAAG AACCCACGAA GCAGGGCGAAG
     TTCTGAGTTT GAATCTTTGA ACTTAATCTA CACCATAAGT TTAGGAATGC ACGGGCGCTTC TGTGTCTGTC GGGGCCATTC TTGGGTGCTT CGTCCGCTTC

101  TTCATTGTTC TCAACATTCT AGCTGCTCTT GCTGCATTTG CTCTGGAATT CTTGTAGAGA TATTACTTGT CCTTCCAGGC TGTTCTTTCT GTAGCTCCT
     AGTTAACAAG AGTTGTAAGA TCGACGAGAA CGACGTAAAC GAGACCTTAA ATAATGAACA GAACATCTCT GGAAGGTCCG ACAAGAAAGA CATCGAGGGA

201  TGTTTTCTTT TTGTGATCAT GTTGCGAGATG GCTGGGCAGT GCTCCCAAAA TGAATATTTT GACAGTTGT CATACCTTGT CAACTTCGAT
     ACAAAAGAAA AACACTAGTA CAACGCTCTAC CGACCCGTCA CGAGGGTTTT ACTTATAAAA CTGTCAAACA GTATGGAACTA GTTGAAGCTA
        Me      tLeuGlnMet AlaGlyGlnC  ysSerGlnAs nGluTyrPhe AspSerLeuL euHisAlaCy sIleProCys GlnLeuArgC
  1

301  GTTCTTCTAA TACTCCTCCT CTAACATGTC AGCGTTATTG TAATGCAAGT CAGTGAAAGG AACGAATGCG ATTCTCTGGA CCTGTTTGGG
     CAAGAAGATT ATGAGGAGGA GATTGTACAG TCGCAATAAC ATTACGTTCA GTCACTTTCC TTGCTTACGC TAAGAGACCT GGACAAACCC
     ysSerSerAs nThrProPro LeuThrCysG lnArgTyrCy sAsnAlaSer erValLysGl yThrAsnAla IleLeuTrpT hrCysLeuGl
 29

401  ACTGAGCTTA ATAATTTCTT TGGCAGTTTT CGTGCTAATG TTTTTGCTAA GGAAGATAAG CTCTGAACCA AGTTAAAAA CACAGGATCA
     TGACTCGAAT TATTAAAGAA ACCGTCAAAA GCACGATTAC AAAAACGATT CCTTCTATTC GAGACTTGGT TCAATTTTTT GTGTCCTAGT
     yLeuSerLeu IleIleSerL euAlaValPh eValLeuMet PheLeuLeuA rgLysIleSe rSerGluPro LeuPheLysAs nThrGlySer
 62

501  GGTCTCCTGG CATTGACCTA GAAAAGAGCA TGAAATTATT TGAAATTATT CTTCCGAGTA GCCTCGAGTA CACGGTGGAA GAATGCACCT
     CCAGAGGACC GTAACTGGAC CTTTCTCGT ACTTTAATAA ACTTTAATAA GAAGGCTCTC CGGAGCTCAT GTGCCACCTT CTTACGTGGA
     GlyLeuLeuG lyMetAlaAs nIleAspLeu LysProLysV pGluIleIle rgThrGlyAs lyLeuGluTy rThrValGlu GluCysThrC
 95

601  GTGAAGACTG CATCAAGAGC AAACCGAAGG TCGACTCTGA CCACTCCCAG CTATGGAGGA AGGCGCAACC ATTCTTGTCA CCACGAAAAC
     CACTTCTGAC GTAGTTCTCG TTTGGCTTCC AGCTGAGACT GGTGAGGGTC GATACCTCCT TCCGCGTTGG TAAGAACAGT GGTGCTTTTG
     ysGluAspCy sIleLysSer LysProLysV alAspSerAs pHisCysPhe laMetGluPro ProLeuProA laThrLysTh rIleLeuValT hrThrLysTh
129

701  GAATGACTAT TGCCAGCTGC TTTGAGTGCT ACGGAGATAG AGAAATCAAT TTCTGCTAGG AGAAGATCC TAATTAACCA TTTCGACTCG AGCAGTGCCA
     CTTACTGATA ACGGTCGACG AAACTCACGA TGCCTCTATC TCTTTAGTTA AGAGACGATCC ATTAATTGGT AAAGCTGAGC TCGTCACGGT
     rAsnAspTyr CysLysSerL euProAlaAl aLeuSerAla ThrGluIleG luLysSerIl eSerAlaArg  OC*
162

801  CTTTAAAAAT CTTTTGTCAG AATAGATGAT GTGTCAGATC TCTTTAGGAT GACTGTATTT TTCAGTTGCC GATACAGCTT TTTGTCCTCT AACTGTGGAA
     GAAATTTTTA GAAAACAGTC TTATCTACTA CACAGTCTAG AGAAATCCTA CTGACATAAA AAGTCAACGG CTATGTCGAA AAACAGGAGA TTGACACCTT

901  ACTCTTTATG TTAGATATAT TTCTCTAGG AGCTTAATGG TACTGTTGGG TAGAAACTTC TTTGTTTCA GACTGTTCACC... CCTGA
     TGAGAAATAC AATCTATATA AAGAGATCCA ATGACAACCC TCGAATTACC ATCTTTGAAG GAACCAAAGT ACTAATTTCA GAAAAAAAAA GGACT
```

Fig. 3

```
  1 ATGGATGACT CCACAGAAAG GGAGCAGTCA CGCCTTACTT CTTGCCTTAA GAAAAGAGAA GAAATGAAAC TGAAGGAGTG TGTTTCCATC CTCCCACGGA
    TACCTACTGA GGTGTCTTTC CCTCGTCAGT GCGGAATGAA GAACGGAATT CTTTTCTCTT ACTTCCTCAC ACAAAGGTAG GAGGGTGCCT
  1  M  D  D  S   T  E  R  E  Q  S   R  L  T  S  C   L  K  K  R  E   E  M  K  L  K   E  C  V  S  I   L  P  R  K

101 AGGAAAGCCC CTCGTCCCGA TCCTCCAAAG ACGGAAAGCT GCTGGCTGCA ACCTTGCTGC TGGCACTGCT GTCTTGCTGC GTCTTCTTCTA
    TCCTTTCGGG GAGCAGGGCT AGGAGGTTTC TGCCTTTCGA CGACCGACGT TGGAACGACG AGGTGCCACC ACAGAAAGAT
 35  E  S  P  S   V  R  S  S  S   K  D  G  K  L   L  A  A  T  L   L  L  A  L  L   S  C  C  L  T   V  V  S  F  Y

201 CCAGGTGGCC GCCCTGCAAG GGGACCTGGC CAGCCTCCGG GCAGAGCTGC AGGGCCACCA CGCGGAGAAG CTGCCAGCAG GAGCAGGAGC CCCAAGGCC
    GGTCCACCGG CGGGACGTTC CCCTGGACCG GTCGGAGGCC CGTCTCGACG TCCCGGTGGT GCGCCTCTTC GACGGTCGTC CTCGTCCTCG GGGGTTCCGG
 68  Q  V  A  A   L  Q  G  D  L   A  S  L  R  A   E  L  Q  G  H   H  A  E  K  L   P  A  G  A  G   A  P  K  A

301 GGCTTGGAGG AAGCTCCAGC TGTCACCGCG GGACTGAAAA TCTTTGAACC ACCAGCTCCA GGAGAAGGCA ACTCCAGTCA GAACAGCCGTG AATAAGCCGTG
    CCGAACCTCC TTCGAGGTCG ACAGTGGCGC CCTGACTTTT AGAAACTTGG TGGTCGAGGT CCTCTTCCGT TGAGGTCAGT CTTGTCGTCT TTATTCGCAC
101  G  L  E  E   A  P  A  V  T   A  G  L  K  I   F  E  P  P  A   P  G  E  G  N   S  S  Q  N  S   R  N  K  R  A

401 CCGTTCAGGG TCCAGAAGAA ACAGTACACT GCAACTGATT GCGAACTGCTT AAGACTCACT CTATACAAAAA GGATCTTACA CATTTGTTCC
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135  V  Q  G  P   E  E  T  V  T   Q  D  C  L  Q   L  I  A  D  S   E  T  P  T  I   Q  K  G  S  Y   T  F  V  P

501 ATGGCTTCTC AGCTTTAAAA GGGGAAGTGC CCTAGAAGAA AAAGAGAATA AAATATTGGT CAAAGAAACT GGTTACTTTT TCAGGTTTTA
    TACCGAAGAG TCGAAATTTT CCCCTTCACG GGATCTTCTT TTTCTCTTAT TTTATAACCA GTTTCTTTGA CCAATGAAAA AGTCCAAAAT
168  W  L  S   F  K  R  G  S   A  L  E  E  K   E  N  K  I  L   V  K  E  T  G   Y  F  F  I  Y   G  Q  V  L

601 TATACTGATA AGACCTACGC CATGGGACAT CTAATTCAGA GGAAGAAGGT CCATGTCTTT GGGGATGAAT TGACTCCTGGT GACTTGTTT CGATGTATTC
    ATATGACTAT TCTGGATGCG GTACCCTGTA GATTAAGTCT CCTTCTTCCA GGTACAGAAA CCCCTACTTA ACTGAGGACCA CTGAAACAAA GCTACATAAG
201  Y  T  D  K   T  Y  A  M  G   H  L  I  Q  R   K  K  V  H   V  F  G  D  E   L  S  L  V  T   L  F  R  C  I  Q

701 AAAATATGCC TGAAACACTA CCCAATAATT CCTGCTATTC AGCTGGCATT GCAAAACTGG AAGAAGGAGA TGAACTCCAA CTTGCAATAC CAAGAGAAA
    TTTTATACGG ACTTTGTGAT GGGTTATTAA GGACGATAAG TCGACCGTAA CGTTTTGACC TTCTTCCTCT ACTTGAGGTT GAACGTTATG GTTCTCTTT
235  N  M  P   E  T  L   P  N  N  S   C  Y  S   A  G  I   A  K  L  E   E  G  D   E  L  Q   L  A  I  P   R  E  N

801 TGCACAAATA TCACTGGATG GAGATGTCAC ATTTTTTGGT GCATTGAAAC TGCTGTGA
    ACGTGTTTAT AGTGACCTAC CTCTACAGTG TAAAAAACCA CGTAACTTTG ACGACACT
268  A  Q  I   S  L  D  G   D  V  T   F  F  G   A  L  K  L   L  Q
```

Fig. 4A

```
1    GGTACGGAGGC TTCCTAGAGG GACTGGGAACC TAATTCTCCT GAGGCTGGAG GAGGGTGGAG GGTCTCAAGG CAACGCTGGC CCCACGACGG AGTGCCAGGA
     CCATGCTCCG AAGGATCTCC CTGACCTTGG ATTAAGAGGA CTCCGACCTC  CCAGGTTCC GTTGCGACCG GGGTGCTGCC TCACGGTCCT

101  GCACTAACAG TACCCTTAGC TTGCTTTCCT CCTCCCTCCT TTTTATTTTC AAGTTCCTTT TTATTCTCC AACCTTCTTCC CTTCTGACCC
     CGTGATTGTC ATGGGAATCG AACGAAAGGA GGAGGGAGGA AAAATAAAAG TTCAAGGAAA AATAAAGAGG TTGGAAGAGG GAAGACGTGG

201  ACTGCCCGTA CCCTTACCCG CCCCGCCACC TCCTTGCTAC CCCACTCTTG AAACCACAGC TGTTGGCAGG GTCCCCAGCT CATGCCAGCC TCATCTCCTT
     TGACGGGCAT GGGAATGGGC GGGGCGGTGG AGGAACGATG GGGTGAGAAC TTTGGTGTCG CAGGGTGTCGA GTACGGTCGG AGTAGAGGAA
                                                                                                        M  P  A  S  S  P  F
  1

301  TCTTGCTAGC CCCCAAAGGG CCTCCAGGCA ACATGGGGGG CCCAGTCAGA GAGCCGGCAC TCTCAGTTGC CCTCTGGTTG AGTTGGGGG CAGCTCTGGG
     AGAACGATCG GGGGTTTCCC GGAGGTCCGT TGTACCCCCC GGGTCAGTCT CTCGGCCGTG AGAGTCAACG GGAGACCAAC TCAACCCCC GTCGAGACCC
     L  L  A    P  K  G    P  P  G  N    M  G  G    P  V  R    E  P  A  L    S  V  A    L  W  L    S  W  G  A    A  L  G
  8

401  GGCCGTGGCT CTCTGCTGAC CTCTGCCATGG CCAACAAACA GAGCTGCAGA GCCTCAGGAG AGAGGTGAGC CGGCTGCAGG CCCCTCCCAG
     CCGGCACCGA GAGACGACTG GAGACGGTACC GGTTGTTTGT CTCGACGTCT CGGAGTCCTC TCTCCACTCG GCCGACGTCC GGGGAGGGTC
     A  V  A    C  A  M  A    L  L  T    Q  Q  T    E  L  Q  S    L  R  R    E  V  S    R  L  Q  G    P  S  Q

501  AATGGGGAAG GGTATCCCTG GCAGAGTCTC CCGGAGCAGA GTTCCGATGC CCTGGAAGCC TGGGAGAATG CCGGAAAAGG AGAGCAGTGC
     TTACCCCTTC CCATAGGGAC CGTCTCGAG GGCCTCGTCT CAAGGCTAGG GGACCTTCGG ACCCTCTTAC GGCCTTTTCC TCTCGTCACG
     N  G  E  G    Y  P  W    Q  S  L    P  E  Q  S    S  D  A    L  E  A    W  E  N  G    E  R  S    R  K  R    R  A  V  L
  74

601  TCACCCAAAA ACAGAAGAAG CAGCACTCTG TCCTGCACCT GGTTCCCATT AACGCCACCT CCAAGGATGA CTCCGATGTG ACAGAGGTGA TGTGGCAACC
     AGTGGGTTTT TGTCTTCTTC GTCGTGAGAC AGGACGTGGA CCAAGGGTAA TTGCGGTGGA GGTTCCTACT GAGGCTACAC TGTCTCCACT ACACCGTTGG
     T  Q  K    Q  K  K    Q  H  S  V    L  H  L    V  P  I    N  A  T  S    K  D  D    S  D  V    T  E  V  M    W  Q  P
  108

701  AGCTCTTAGG CGTGGGAGAG GCCTACAGGC CCAAGGATAT GGTGTCCGAA TCCAGGATGC GGAGTTTAT GCCCAGTCCT GTTTCAGAC
     TCGAGAATCC GCACCCTCTC CGGATGTCCG GGTTCCTATA CCACAGGCTT AGGTCCTACG ACCTCAAATA CGGGTCAGGA CAAAGTTCTG
     A  L  R    R  G  R  G    L  Q  A    Q  G  Y    G  V  R  I    Q  D  A    G  V  Y    L  L  Y  S    Q  V  L    F  Q  D
  141

801  GTGACTTTCA CCATGGGTCA GGTGGTGTCT CGAGAAGGCA AAGGAAGGCA GGAGACTCTA TTCCGATGTA TAAGAAGTAT GCCCTCCCAC CCGGACCGGG
     CACTGAAAGT GGTACCCAGT CCACCACAGA GCTCTTCCGT TTCCTTCCGT CCTCTGAGAT AAGGCTACAT ATTCTTCATA CGGGAGGGTG GGCCTGGCCC
     V  T  F  T    M  G  Q    V  V  S    R  E  G  Q    K  G  R  Q    E  T  L    F  R  C  I    R  S  M    P  D  R  A
  174

901  CCTCAACACAG CTGCTATAGC GCAAGGTGTCT TCCATTTACA CCAAGGGGAT ATTCTGAGTG GCGAAACTTA ACCTCTCTCC
     GGATGTTGTC GACGATATCG GCGTTCCACAGA AGGTAAATGT GGTTCCCCTA TAAGACTCAC CGCTTTGAAT TGGAGAGAGG
     Y  N  S    C  Y  S    A  G  V  F    H  L  H    Q  G  D    I  L  S  V    I  I  P    R  A  R    A  K  L  N    L  S  P
  208
```

```
1001  ACATGGAACC TTCCTGGGGT TTGTGAAACT GTGATTGTGT TATAAAAAGT GGCTCCCAGC TTGGAAGACC AGGGTGGGTA CATACTGGAG ACAGCCAAGA
      TGTACCTTGG AAGGACCCCA AACACTTTGA CACTAACACA ATATTTTTCA CCGAGGGTCG AACCTTCTGG TCCCACCCAT GTATGACCTC TGTCGGTTCT
 241   H   G   T   F   L   G   F   V   K   L   O

1101  GCTGAGTATA TAAAGGAGAG GGAATGTGCA CATCTTCCTG GGTTGGCTC CCCGTTCCTC.ACTTTTCCCT TTTCATTCCC ACCCCCTAGA
      CGACTCATAT ATTTCCTCTC CCTTACACGT GTAGAAGGAC CCAAACCGAG GGGCAAGGAG TGAAAGGGA AAAGTAAGGG TGGGGGATCT

1201  CTTTGATTTT ACGGATATCT TGCTTCTGTT CCCCATGGAG CTCCGAATTC TTGGGTGTGT GTAGATGAGG GGCGGGGGAC CATTGTTCAG
      GAAACTAAAA TGCCTATAGA ACGAGACAA GGGGTACCTC GAGGCTTAAG AACGCACACA CATCTACTCC CCGCCCCCTG GTAACAAGTC

1301  ACCTGGTCGG GGCCCACTGG AAGCATCCAG AACAGCACCA CCATCTTA
      TGGACCAGCC CCGGGTGACC TTCGTAGGTC TTGTCGTGGT GGTAGAAT
```

Fig. 48

FIGURE 5A

PRO   XXXXXXXXXXXXXXX   (Length = 15 amino acids)

Comparison Protein   XXXXXYYYYYYY   (Length = 12 amino acids)
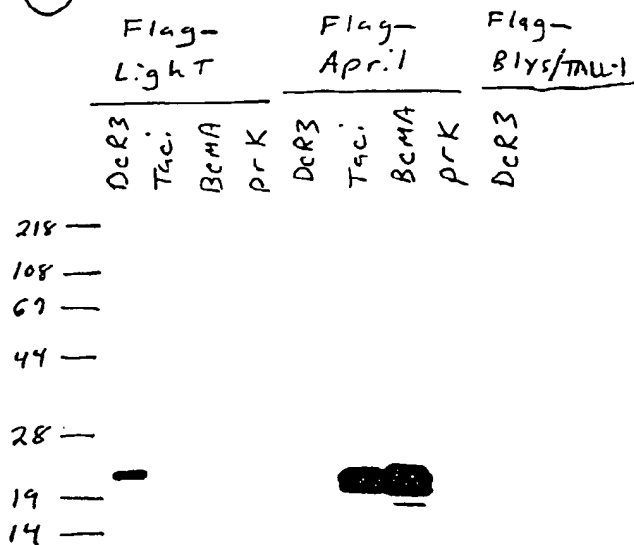
% amino acid sequence identity =

(the number of identically matching amino acid residues between the two polypeptide sequences as determined by ALIGN-2) divided by (the total number of amino acid residues of the PRO polypeptide) =
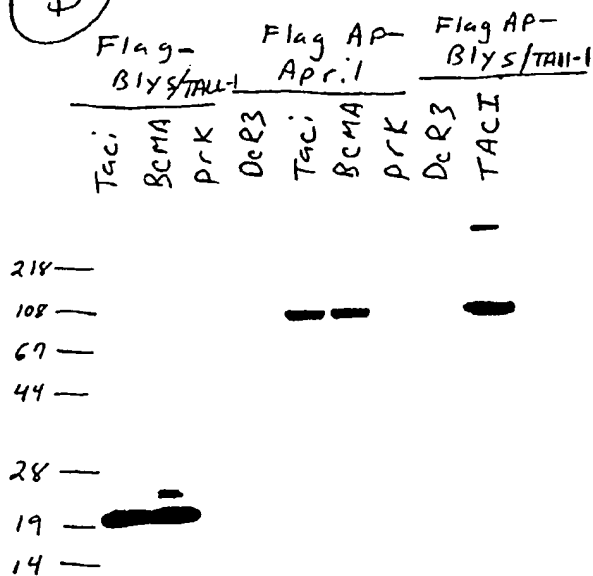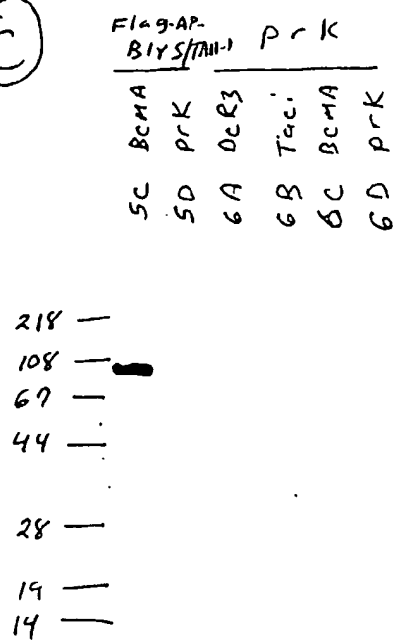
5 divided by 15 = 33.3%

FIGURE 5B

PRO   XXXXXXXXXX   (Length = 10 amino acids)

Comparison Protein   XXXXXYYYYYYYZZYZ   (Length = 15 amino acids)

% amino acid sequence identity =

(the number of identically matching amino acid residues between the two polypeptide sequences as determined by ALIGN-2) divided by (the total number of amino acid residues of the PRO polypeptide) =
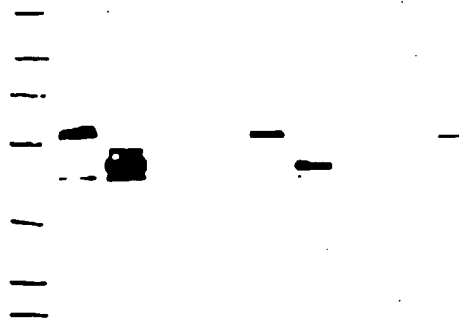
5 divided by 10 = 50%

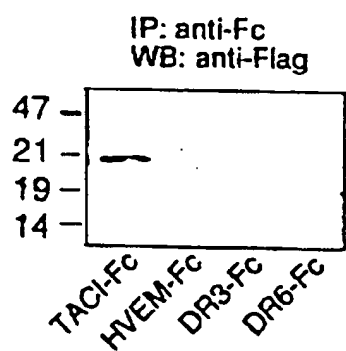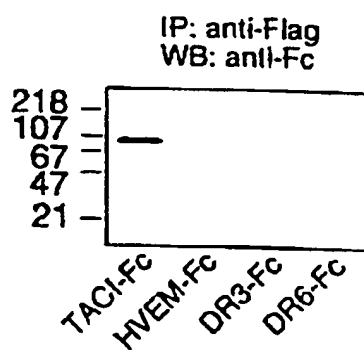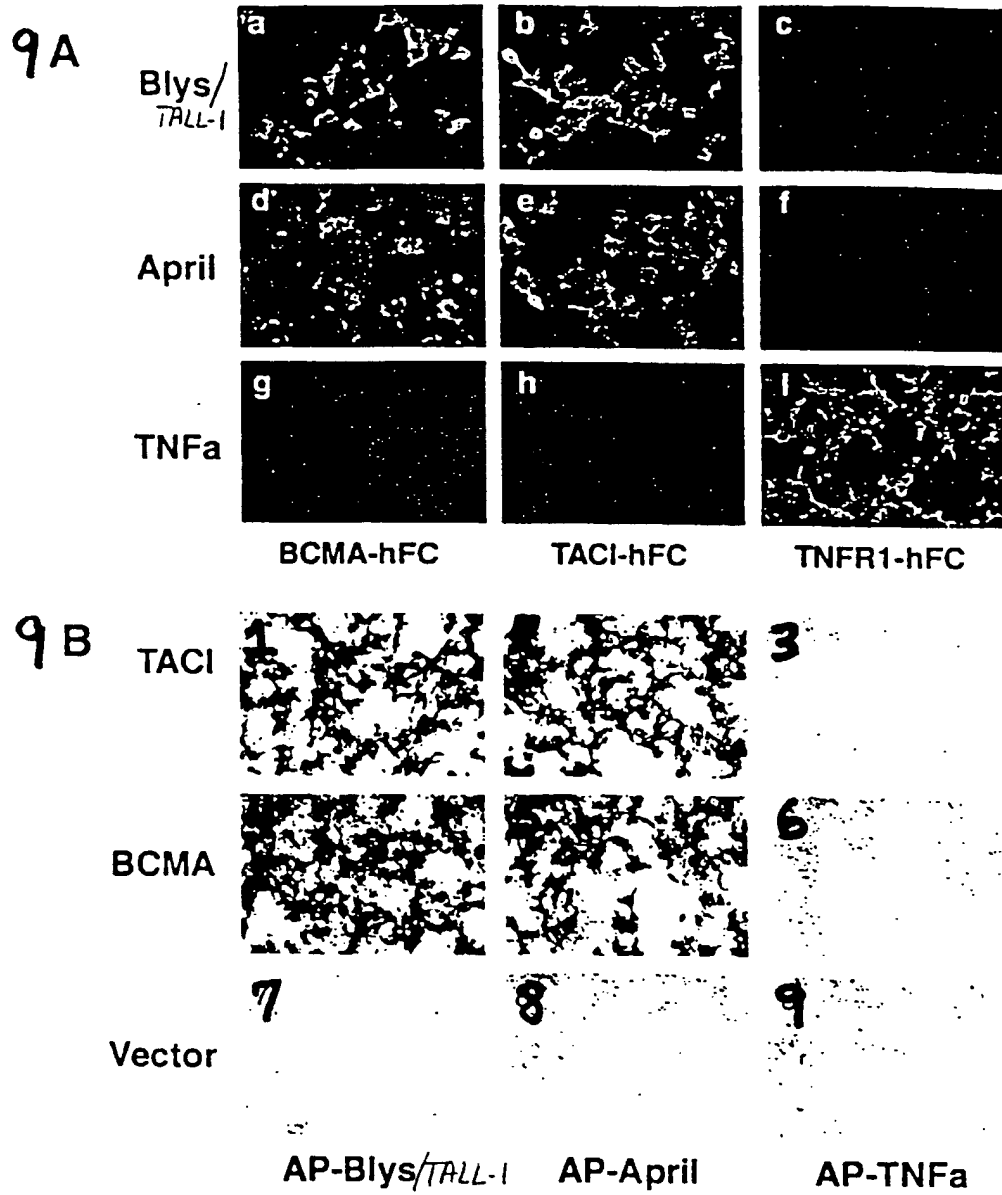Fig. 6

Fig. 7
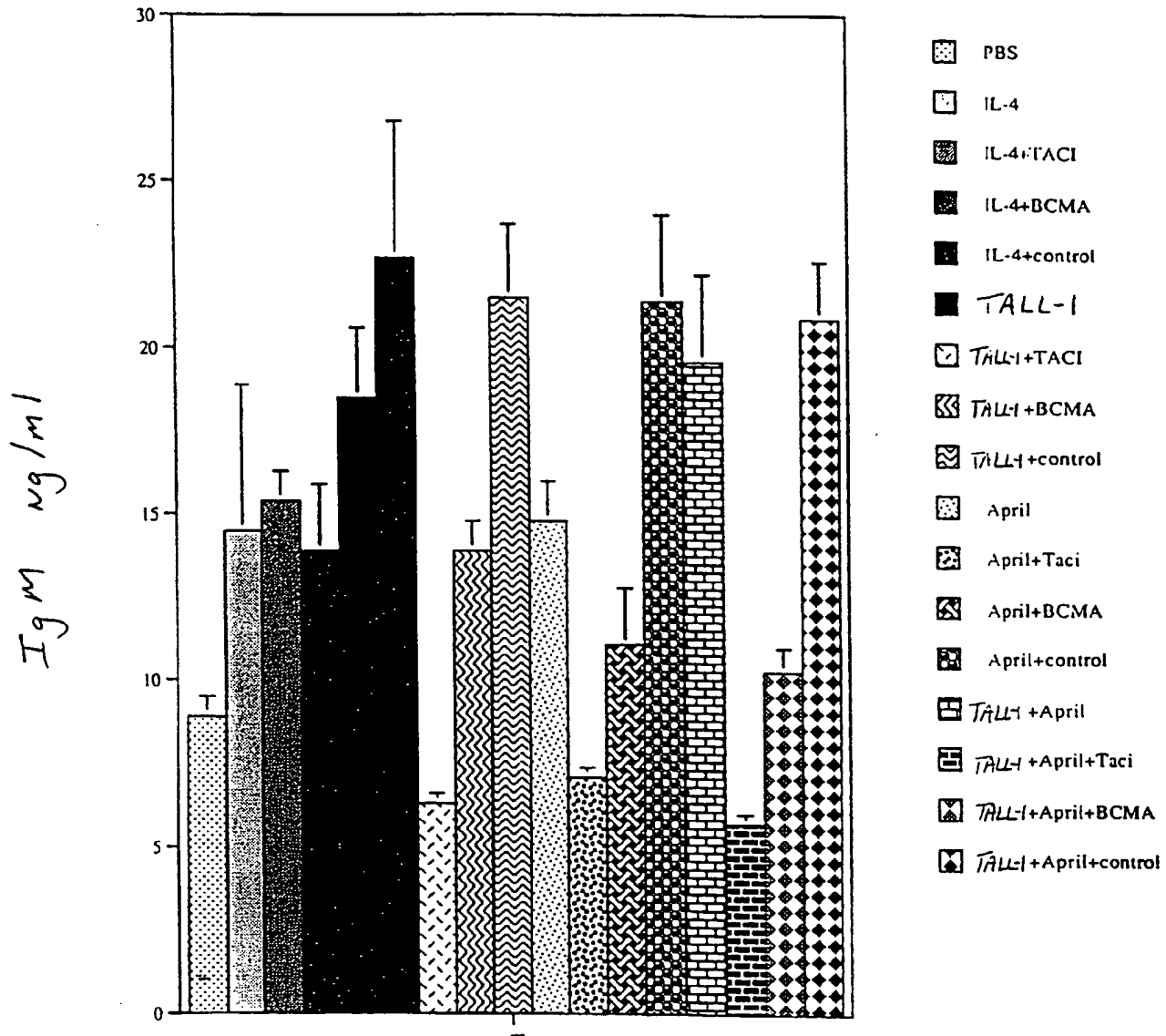
Fig. 8

Ip: with
α Flag
WB: with
α Hu IgG. HRP

Fig. 8G

Fig. 8H

Fig. 9

Fig. 10
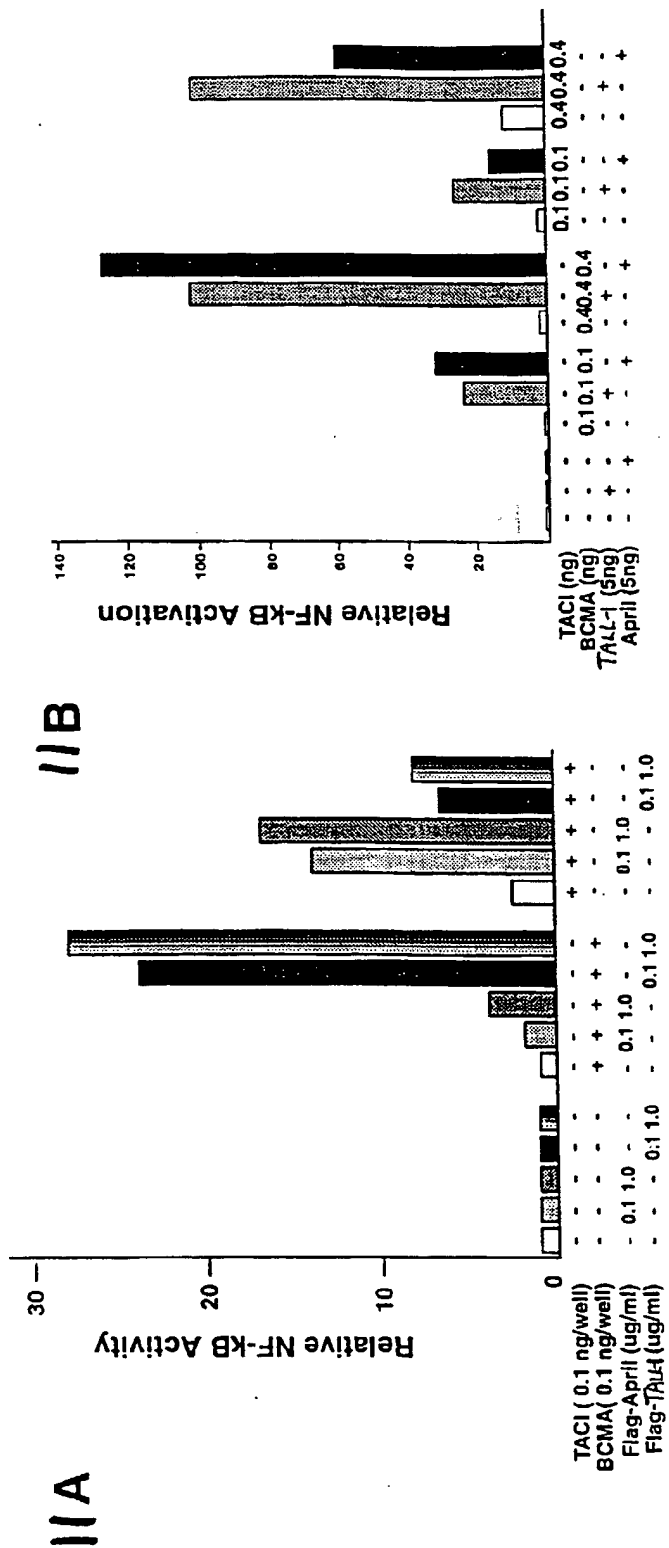
Fig. 11

Fig. 11C
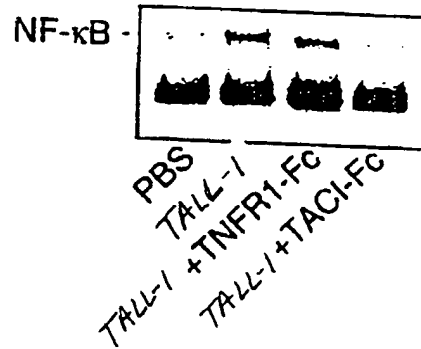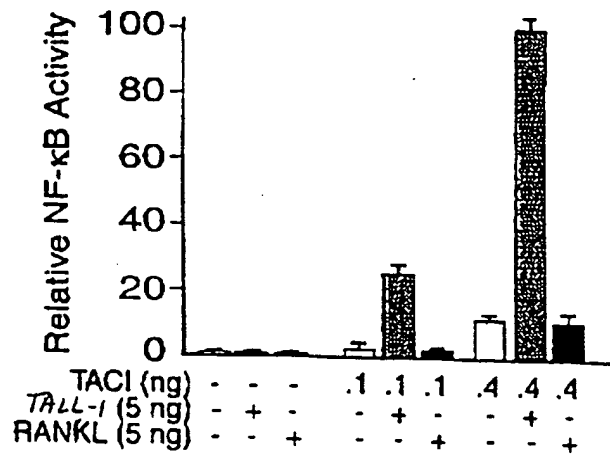
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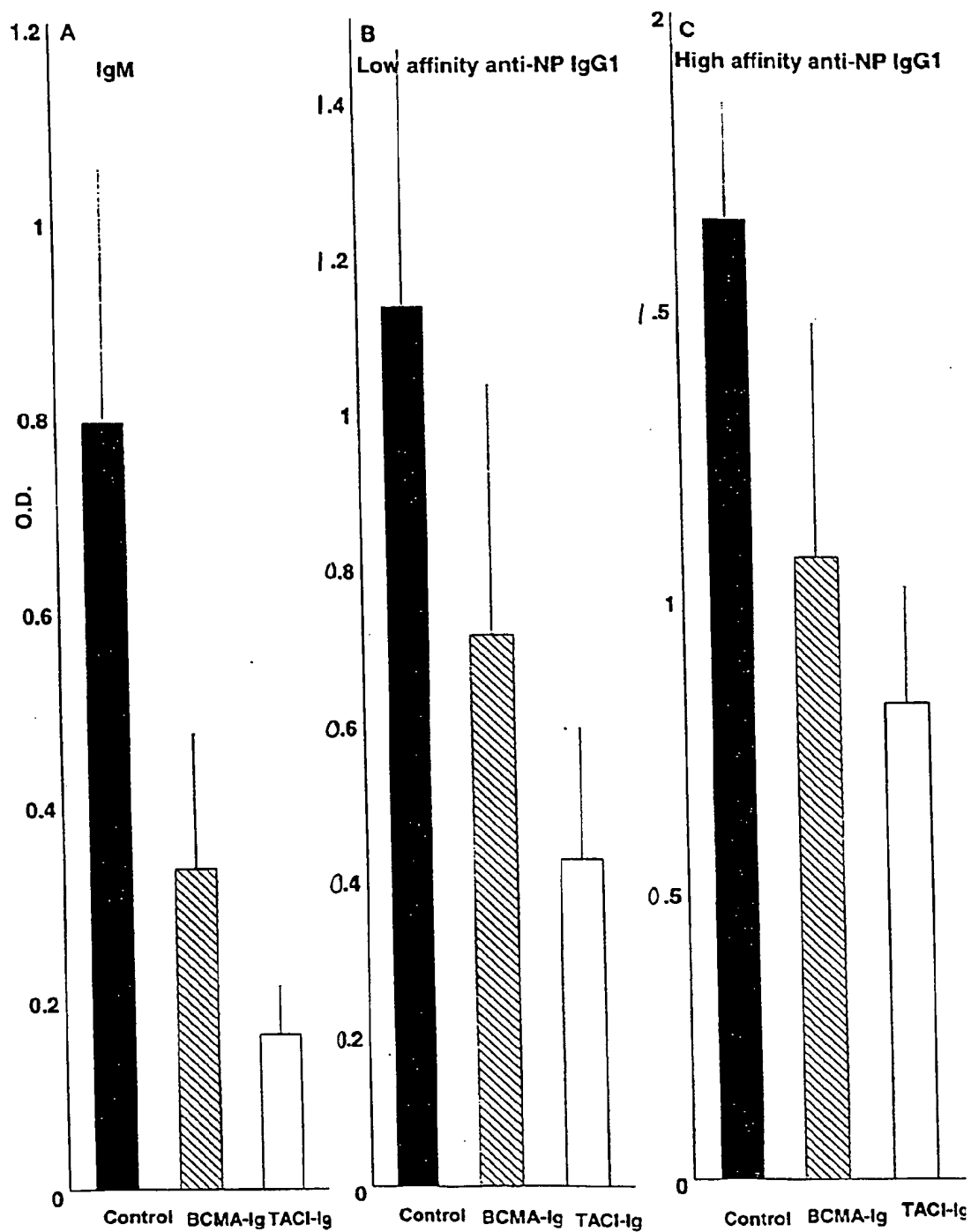
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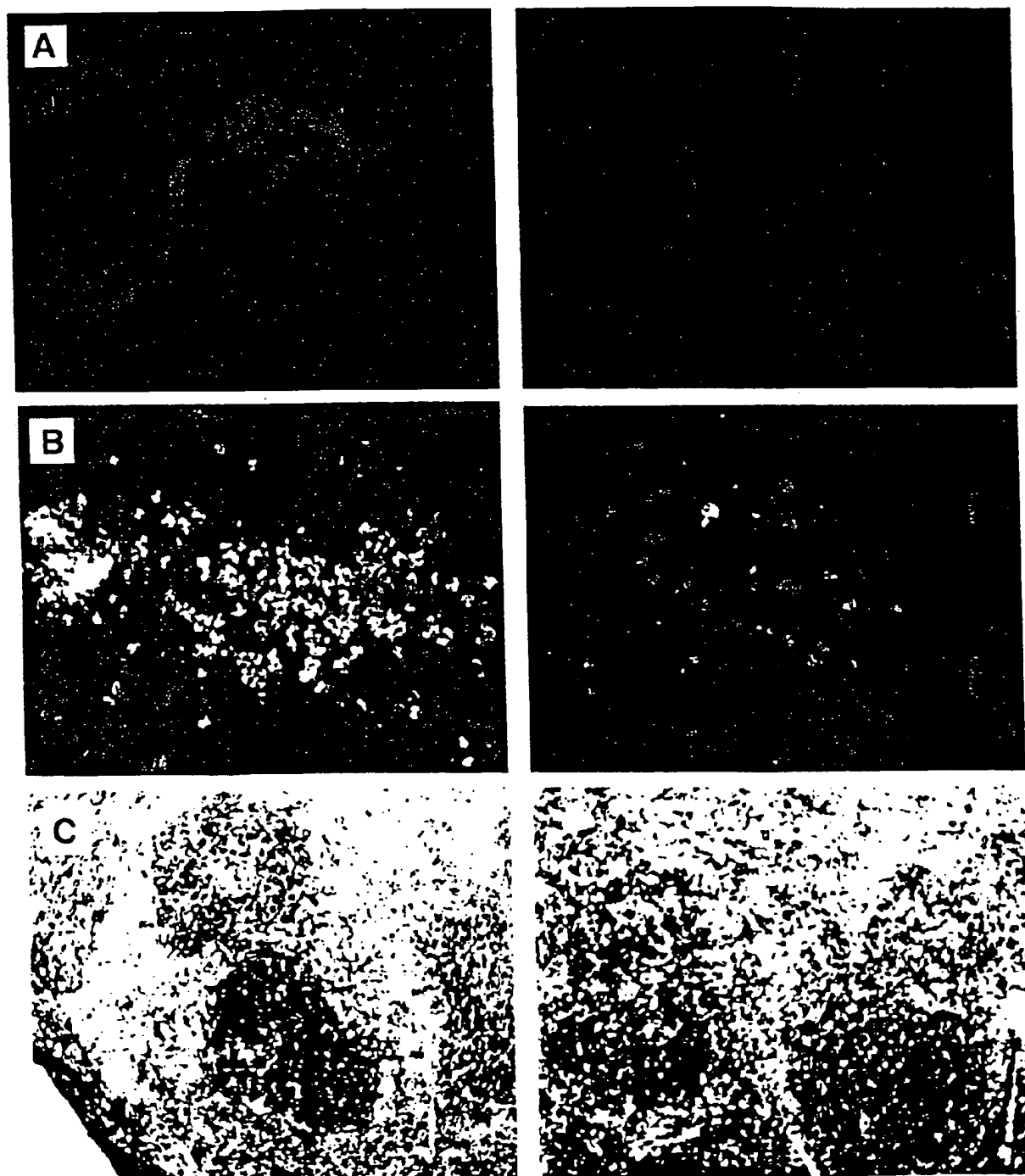
Fig. 13-1
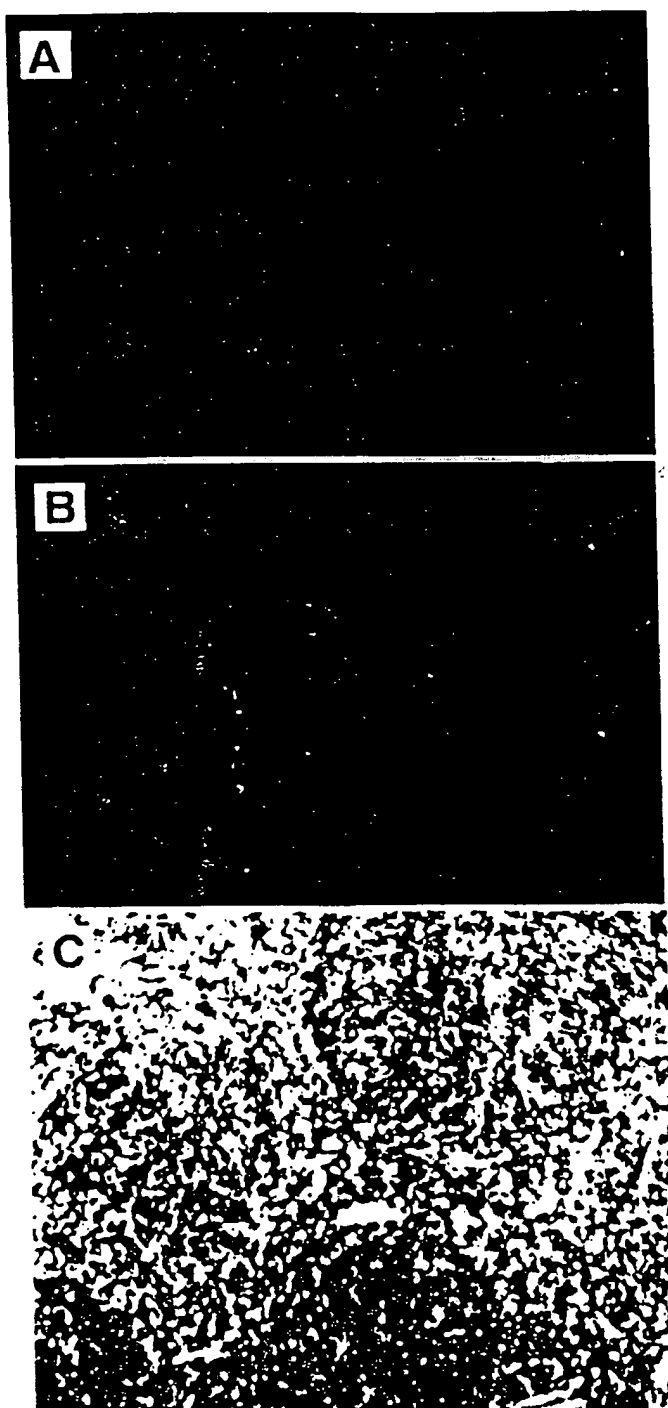
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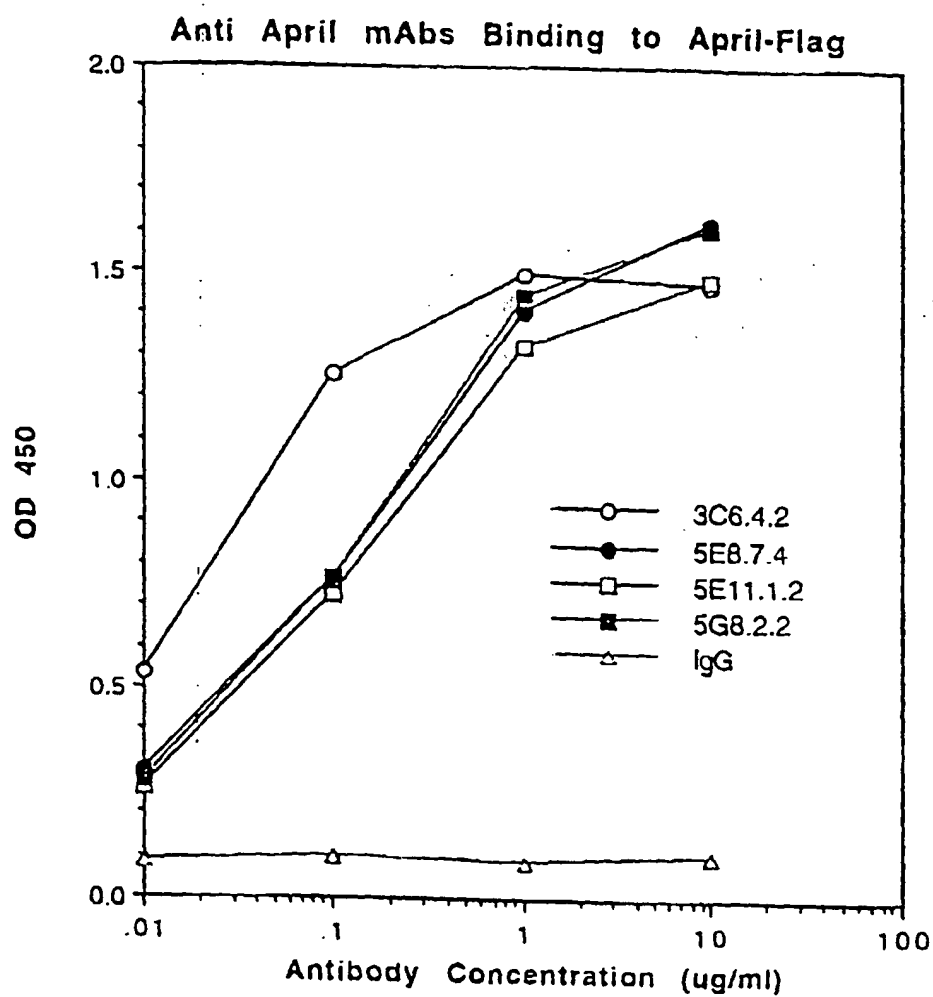
Fig. 14A

Summary of antiApril mABs

mAb	Isotype	Epitope	Binding (ELISA)		Block April bindiong to	
			April	TALL	BCMA	TACI
3C6.4.2	IgG2a	C	++	-	+++	+
5E8.7.4	IgG2a	A	++	-	-	-
5E11.1.2	IgG1	C?	++	-	+	+
5G8.2.2	IgG2a	B	++	-	-	-

MAb 5E11.1.2 may bind to the similar epitope.

Fig. 14B

Fig. 15

Fig. 16A

Fig. 16B
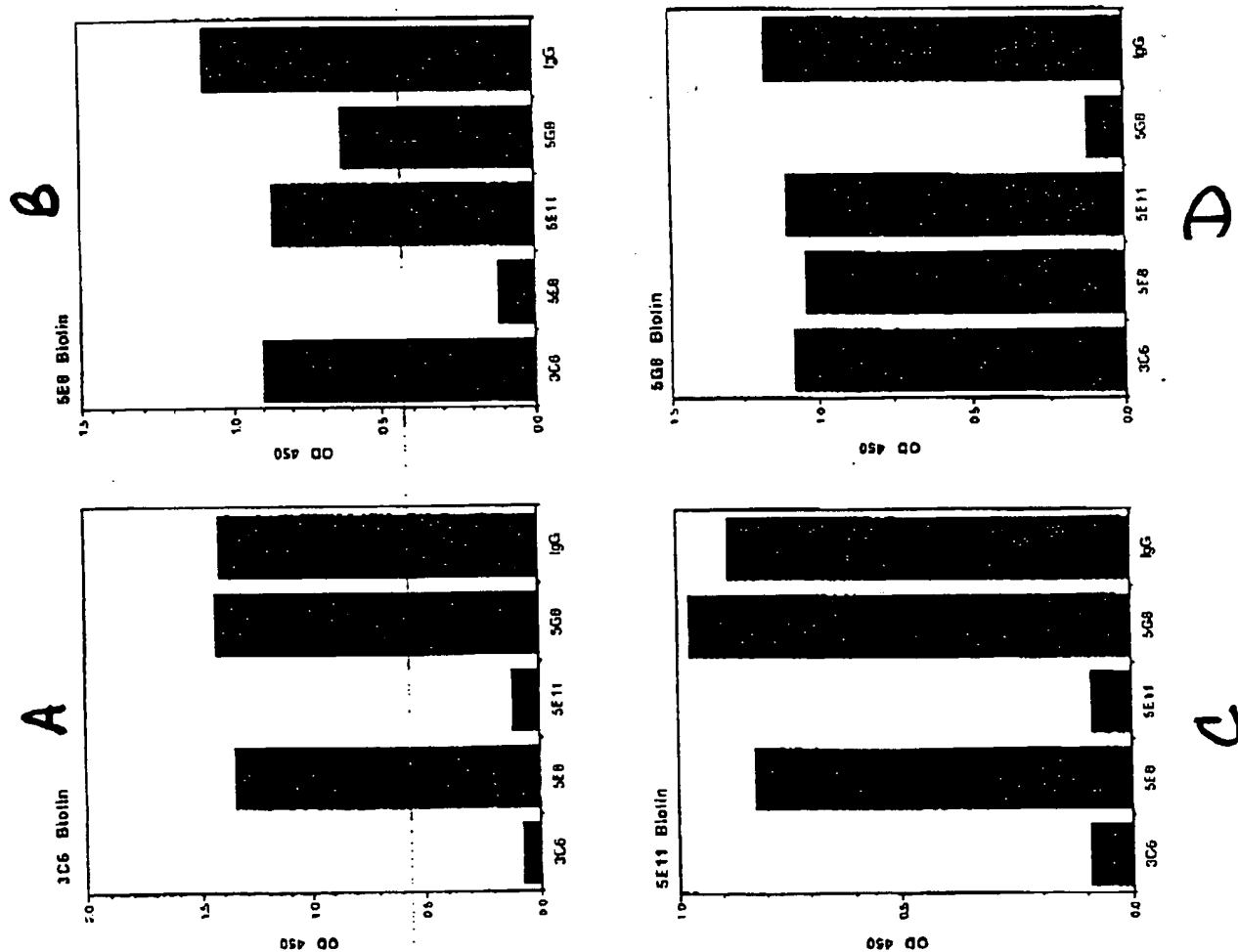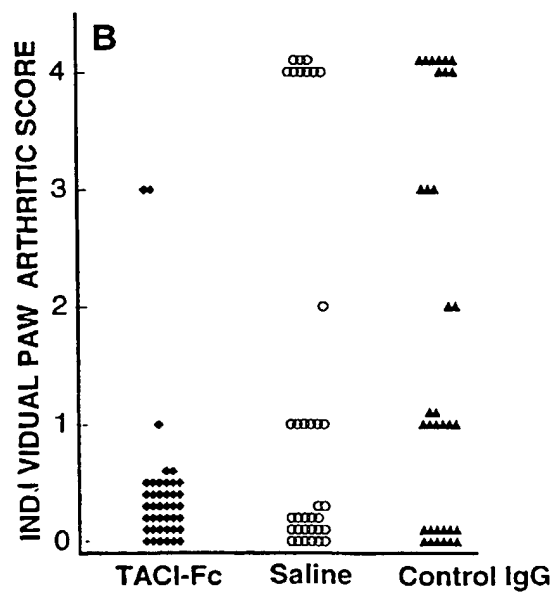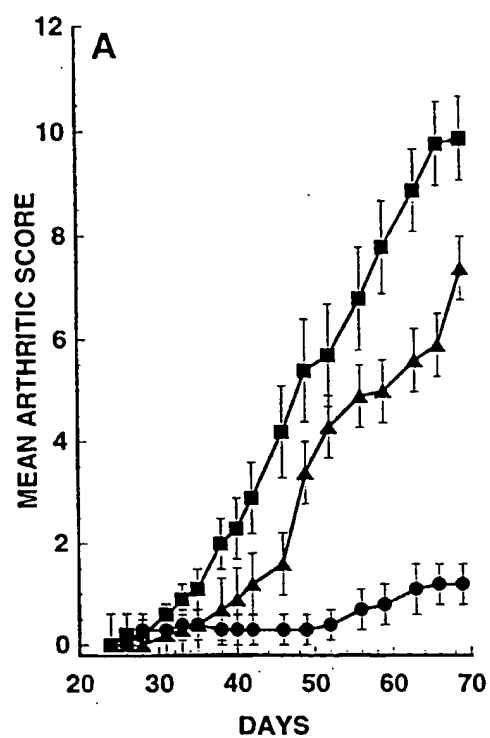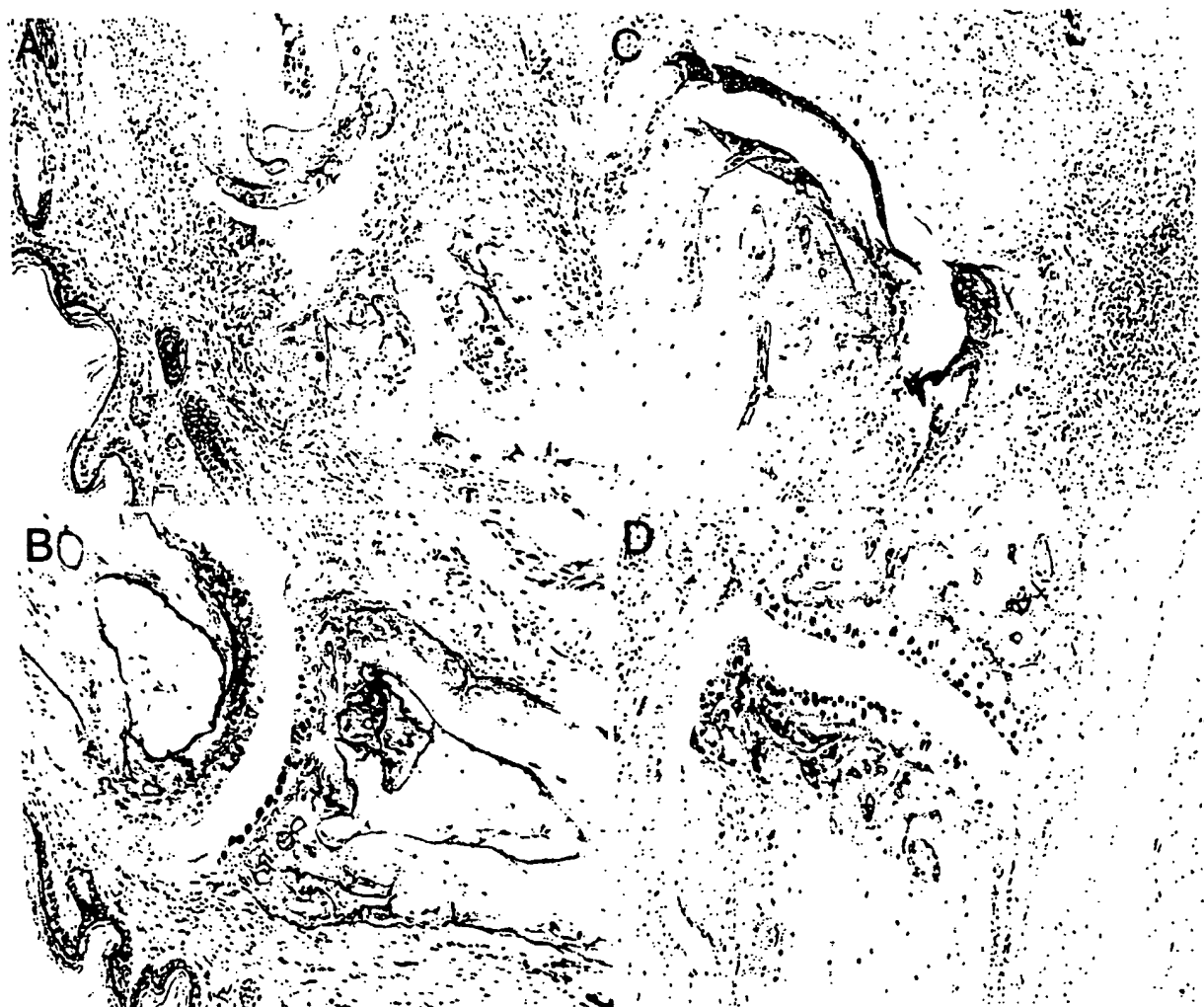
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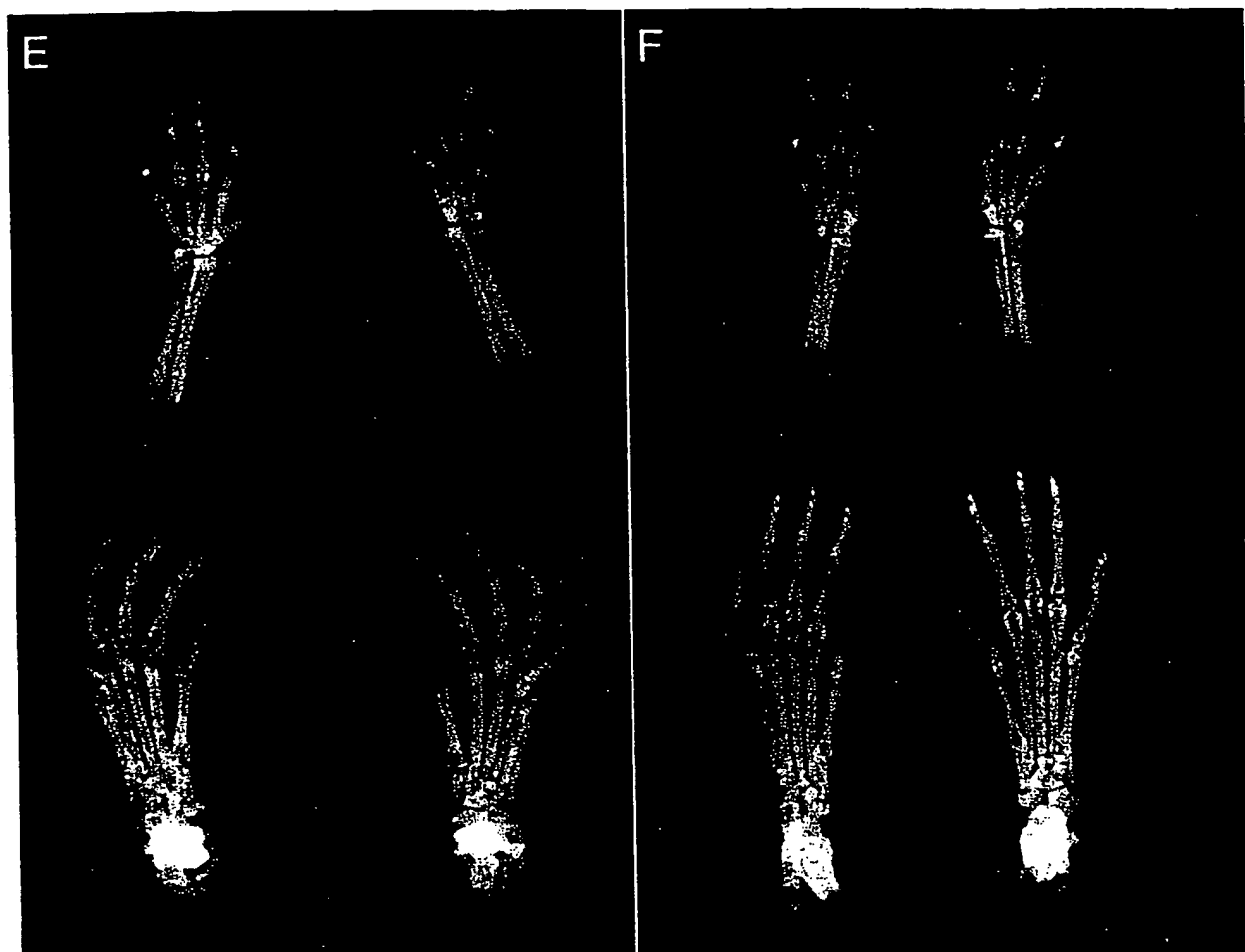
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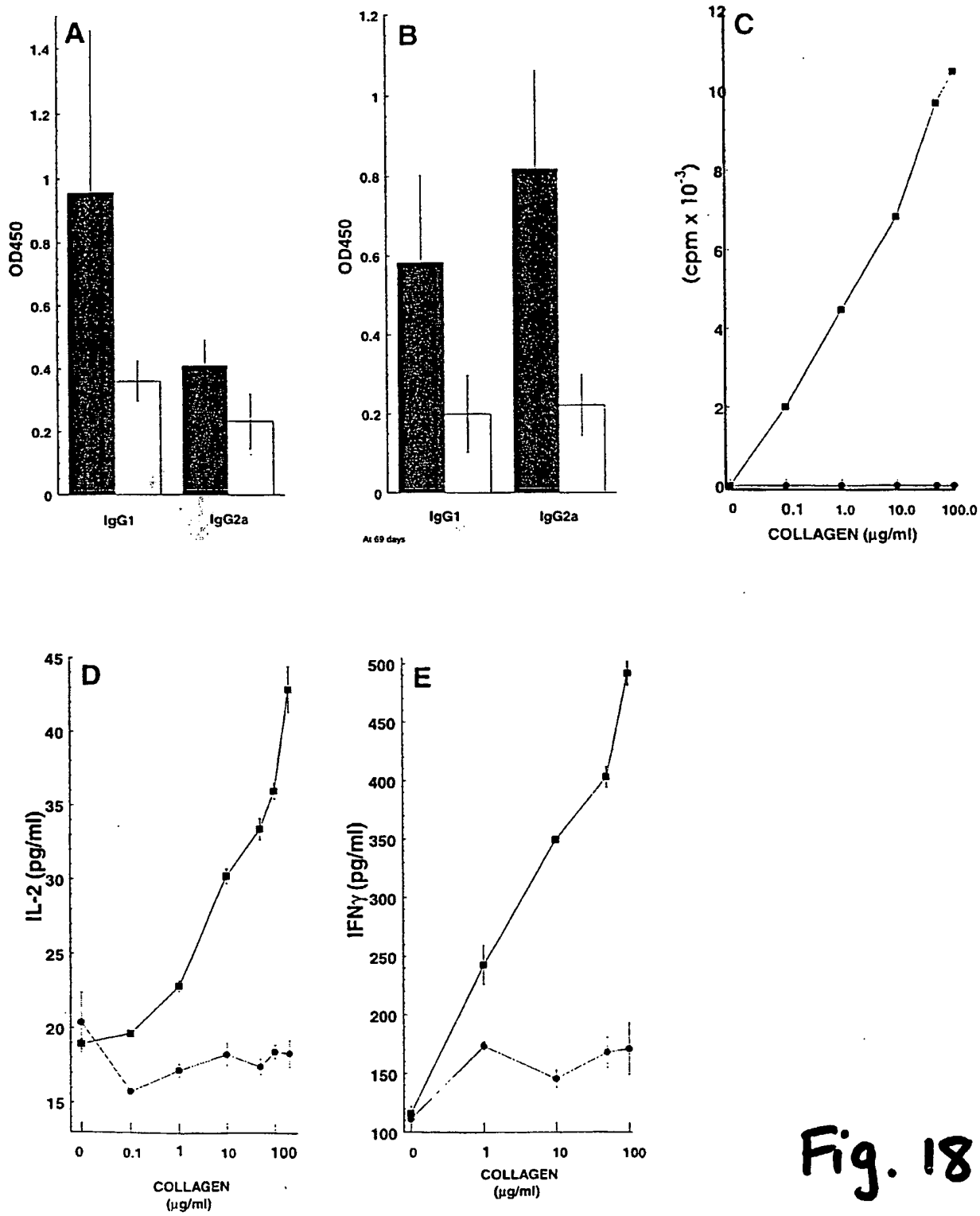
Fig. 18
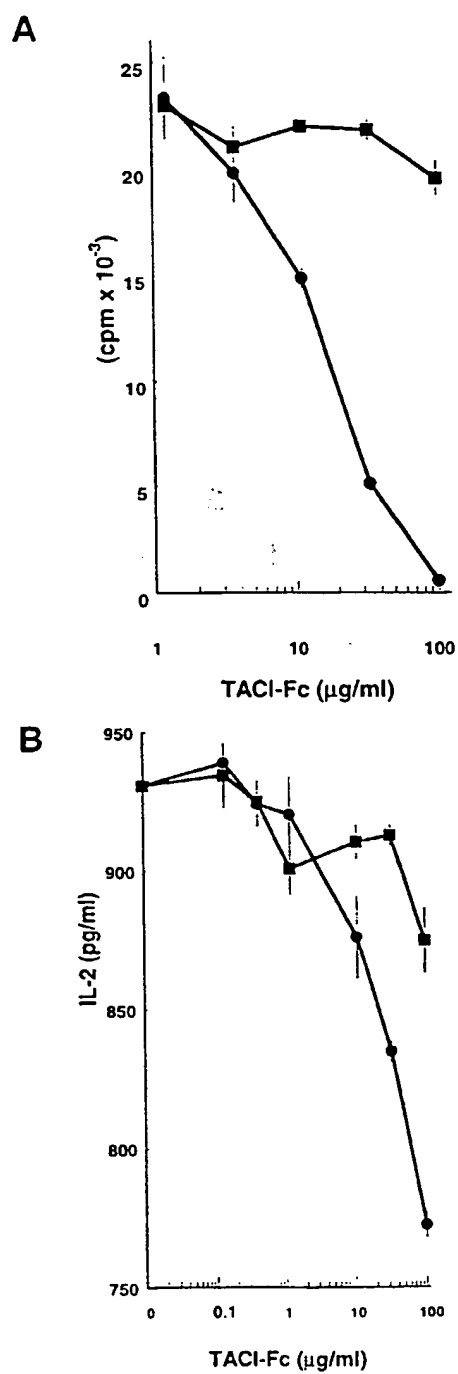
Fig. 19

TACI-Fc treatment of mice inhibits EAE in MBP-TCR transgenic mice
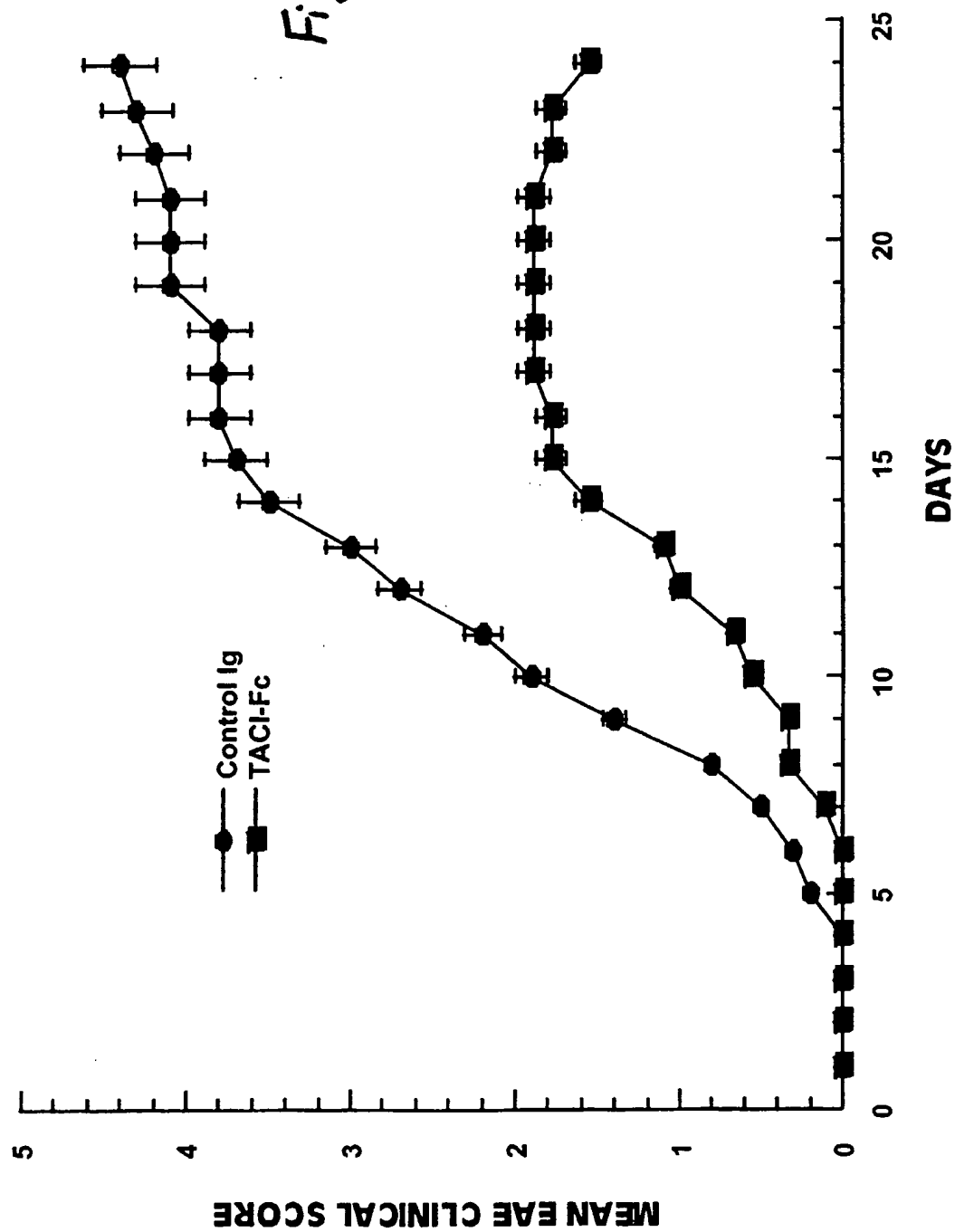
Fig. 20

Sequence Listing

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  cagccttctg caggtcactc agctgccgca aggagcaagg caagttctat 250

  gaccatctcc tgagggactg catcagctgt gcctccatct gtggacagca 300

  ccctaagcaa tgtgcatact tctgtgagaa caagctcagg agcccagtga 350

  accttccacc agagctcagg agacagcgga gtggagaagt tgaaaacaat 400

  tcagacaact cgggaaggta ccaaggattg gagcacagag gctcagaagc 450

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  tctacagcac gctggggctc tgcctgtgtg ccgtcctctg ctgcttcctg 550

  gtggcggtgg cctgcttcct caagaagagg ggggatccct gctcctgcca 600

  gccccgctca aggccccgtc aaagtccggc caagtcttcc caggatcacg 650

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         aggaaagaga caggcagaga aggagagagg cagagaggga gagaggcaga 1150

         gagggagaga ggcagagaga cagagaggga gagagggaca gagagagata 1200

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         cttcctcaaa aaaaaaaaaa aaaaaaa 1377
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    <213> Homo sapiens

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                      20   25   30
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      Met Arg Ser Cys Pro Glu Glu Gln Tyr Trp Asp Pro Leu Leu Gly
                      35   40   45

      Thr Cys Met Ser Cys Lys Thr Ile Cys Asn His Gln Ser Gln Arg
35   50   55   60

      Thr Cys Ala Ala Phe Cys Arg Ser Leu Ser Cys Arg Lys Glu Gln
                      65   70   75

40   Gly Lys Phe Tyr Asp His Leu Leu Arg Asp Cys Ile Ser Cys Ala
                      80   85   90

      Ser Ile Cys Gly Gln His Pro Lys Gln Cys Ala Tyr Phe Cys Glu
                      95   100   105
45
      Asn Lys Leu Arg Ser Pro Val Asn Leu Pro Pro Glu Leu Arg Arg
                      110   115   120

50   Gln Arg Ser Gly Glu Val Glu Asn Asn Ser Asp Asn Ser Gly Arg
                      125   130   135

      Tyr Gln Gly Leu Glu His Arg Gly Ser Glu Ala Ser Pro Ala Leu
                      140   145   150

55   Pro Gly Leu Lys Leu Ser Ala Asp Gln Val Ala Leu Val Tyr Ser
                      155   160   165

      Thr Leu Gly Leu Cys Leu Cys Ala Val Leu Cys Cys Phe Leu Val
                      170   175   180
60
      Ala Val Ala Cys Phe Leu Lys Lys Arg Gly Asp Pro Cys Ser Cys
                      185   190   195
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Gln Pro Arg Ser Arg Pro Arg Gln Ser Pro Ala Lys Ser Ser Gln
                200             205             210
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```
Asp His Ala Met Glu Ala Gly Ser Pro Val Ser Thr Ser Pro Glu
                215             220             225
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```
Pro Val Glu Thr Cys Ser Phe Cys Phe Pro Glu Cys Arg Ala Pro
                230             235             240
```

```
Thr Gln Glu Ser Ala Val Thr Pro Gly Thr Pro Asp Pro Thr Cys
                245             250             255
```

```
Ala Gly Arg Trp Gly Cys His Thr Arg Thr Thr Val Leu Gln Pro
                260             265             270
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Cys Pro His Ile Pro Asp Ser Gly Leu Gly Ile Val Cys Val Pro
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Ala Gln Glu Gly Gly Pro Gly Ala
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<212> DNA
<213> Homo sapiens
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3

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<212> PRT
<213> Homo sapiens

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Ser Leu Leu His Ala Cys Ile Pro Cys Gln Leu Arg Cys Ser Ser
                20   25   30

Asn Thr Pro Pro Leu Thr Cys Gln Arg Tyr Cys Asn Ala Ser Val
                35   40   45

Thr Asn Ser Val Lys Gly Thr Asn Ala Ile Leu Trp Thr Cys Leu
                50   55   60

Gly Leu Ser Leu Ile Ile Ser Leu Ala Val Phe Val Leu Met Phe
                65   70   75

Leu Leu Arg Lys Ile Ser Ser Glu Pro Leu Lys Asp Glu Phe Lys
                80   85   90

Asn Thr Gly Ser Gly Leu Leu Gly Met Ala Asn Ile Asp Leu Glu
                95   100   105

Lys Ser Arg Thr Gly Asp Glu Ile Ile Leu Pro Arg Gly Leu Glu
                110   115   120

Tyr Thr Val Glu Glu Cys Thr Cys Glu Asp Cys Ile Lys Ser Lys
                125   130   135

Pro Lys Val Asp Ser Asp His Cys Phe Pro Leu Pro Ala Met Glu
                140   145   150

Glu Gly Ala Thr Ile Leu Val Thr Thr Lys Thr Asn Asp Tyr Cys
                155   160   165

Lys Ser Leu Pro Ala Ala Leu Ser Ala Thr Glu Ile Glu Lys Ser
                170   175   180

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<211> 858
<212> DNA
<213> Homo sapiens

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aggaaagccc ctctgtccga tcctccaaag acggaaagct gctggctgca 150

accttgctgc tggcactgct gtcttgctgc ctcacggtgg tgtctttcta 200

4

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gcaaaactgg aagaaggaga tgaactccaa cttgcaatac aagagaaaaa 800

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<210> 6
<211> 285
<212> PRT
<213> Homo sapiens

<400> 6
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                20                  25                  30

Leu Pro Arg Lys Glu Ser Pro Ser Val Arg Ser Ser Lys Asp Gly
                35                  40                  45

Lys Leu Leu Ala Ala Thr Leu Leu Leu Ala Leu Leu Ser Cys Cys
                50                  55                  60

Leu Thr Val Val Ser Phe Tyr Gln Val Ala Ala Leu Gln Gly Asp
                65                  70                  75

Leu Ala Ser Leu Arg Ala Glu Leu Gln Gly His His Ala Glu Lys
                80                  85                  90

Leu Pro Ala Gly Ala Gly Ala Pro Lys Ala Gly Leu Glu Glu Ala
                95                  100                 105

Pro Ala Val Thr Ala Gly Leu Lys Ile Phe Glu Pro Pro Ala Pro
                110                 115                 120

Gly Glu Gly Asn Ser Ser Gln Asn Ser Arg Asn Lys Arg Ala Val
                125                 130                 135

Gln Gly Pro Glu Glu Thr Val Thr Gln Asp Cys Leu Gln Leu Ile
                140                 145                 150
```

5

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Ala Asp Ser Glu Thr Pro Thr Ile Gln Lys Gly Ser Tyr Thr Phe
            155                 160                 165

Val Pro Trp Leu Leu Ser Phe Lys Arg Gly Ser Ala Leu Glu Glu
            170                 175                 180

Lys Glu Asn Lys Ile Leu Val Lys Glu Thr Gly Tyr Phe Phe Ile
            185                 190                 195

Tyr Gly Gln Val Leu Tyr Thr Asp Lys Thr Tyr Ala Met Gly His
            200                 205                 210

Leu Ile Gln Arg Lys Lys Val His Val Phe Gly Asp Glu Leu Ser
            215                 220                 225

Leu Val Thr Leu Phe Arg Cys Ile Gln Asn Met Pro Glu Thr Leu
            230                 235                 240

Pro Asn Asn Ser Cys Tyr Ser Ala Gly Ile Ala Lys Leu Glu Glu
            245                 250                 255

Gly Asp Glu Leu Gln Leu Ala Ile Pro Arg Glu Asn Ala Gln Ile
            260                 265                 270

Ser Leu Asp Gly Asp Val Thr Phe Phe Gly Ala Leu Lys Leu Leu
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<211> 1348
<212> DNA
<213> Homo sapiens

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actgcccgta cccttacccg ccccgccacc tccttgctac cccactcttg 250

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<211> 250
<212> PRT
<213> Homo sapiens

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Gly Asn Met Gly Gly Pro Val Arg Glu Pro Ala Leu Ser Val Ala
            20   25   30

Leu Trp Leu Ser Trp Gly Ala Ala Leu Gly Ala Val Ala Cys Ala
            35   40   45

Met Ala Leu Leu Thr Gln Gln Thr Glu Leu Gln Ser Leu Arg Arg
            50   55   60

Glu Val Ser Arg Leu Gln Gly Thr Gly Gly Pro Ser Gln Asn Gly
            65   70   75

Glu Gly Tyr Pro Trp Gln Ser Leu Pro Glu Gln Ser Ser Asp Ala
            80   85   90

Leu Glu Ala Trp Glu Asn Gly Glu Arg Ser Arg Lys Arg Arg Ala
            95   100   105

Val Leu Thr Gln Lys Gln Lys Lys Gln His Ser Val Leu His Leu
            110   115   120

Val Pro Ile Asn Ala Thr Ser Lys Asp Asp Ser Asp Val Thr Glu
            125   130   135

Val Met Trp Gln Pro Ala Leu Arg Arg Gly Arg Gly Leu Gln Ala
            140   145   150

Gln Gly Tyr Gly Val Arg Ile Gln Asp Ala Gly Val Tyr Leu Leu
            155   160   165

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        Tyr Ser Gln Val Leu Phe Gln Asp Val Thr Phe Thr Met Gly Gln
                        170             175             180

  5     Val Val Ser Arg Glu Gly Gln Gly Arg Gln Glu Thr Leu Phe Arg
                        185             190             195

        Cys Ile Arg Ser Met Pro Ser His Pro Asp Arg Ala Tyr Asn Ser
                        200             205             210
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        Cys Tyr Ser Ala Gly Val Phe His Leu His Gln Gly Asp Ile Leu
                        215             220             225

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                        230             235             240

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 30                     20              25              30

        Met Arg Ser Cys Pro Glu Glu Gln Tyr Trp Asp Pro Leu Leu Gly
                        35              40              45

 35     Thr Cys Met Ser Cys Lys Thr Ile Cys Asn His Gln Ser Gln Arg
                        50              55              60

        Thr Cys Ala Ala Phe Cys Arg Ser Leu Ser Cys Arg Lys Glu Gln
                        65              70              75
 40
        Gly Lys Phe Tyr Asp His Leu Leu Arg Asp Cys Ile Ser Cys Ala
                        80              85              90

        Ser Ile Cys Gly Gln His Pro Lys Gln Cys Ala Tyr Phe Cys Glu
 45                     95              100             105

        Asn Lys Leu Arg Ser Pro Val Asn Leu Pro Pro Glu Leu Arg Arg
                        110             115             120

 50     Gln Arg Ser Gly Glu Val Glu Asn Asn Ser Asp Asn Ser Gly Arg
                        125             130             135

        Tyr Gln Gly Leu Glu His Arg Gly Ser Glu Ala Ser Pro Ala Leu
                        140             145             150
 55  .
        Pro Gly Leu Lys Leu Ser Ala Asp Gln Val Ala Leu Val Tyr Ser
                        155             160             165

        Thr Leu Gly Leu Cys Leu Cys Ala Val Leu Cys Cys Phe Leu Val
 60                     170             175             180

        Ala Val Ala Cys Phe Leu Lys Lys Arg Gly Asp Pro Cys Ser Cys
                        185             190             195
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     Gln Pro Arg Ser Arg Pro Arg Gln Ser Pro Ala Lys Ser Ser Gln
                     200                 205                 210

 5   Asp His Ala Met Glu Ala Gly Ser Pro Val Ser Thr Ser Pro Glu
                     215                 220                 225

     Pro Val Glu Thr Cys Ser Phe Cys Phe Pro Glu Cys Arg Ala Pro
                     230                 235                 240
10
     Thr Gln Glu Ser Ala Val Thr Pro Gly Thr Pro Asp Pro Thr Cys
                     245                 250                 255

     Ala Gly Arg Thr Ala Pro Pro Arg Glu Gly
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     <220>
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60
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<212> DNA
<213> Homo sapiens

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                                         11

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       tcagcctcag gagaattagg ttccagtccc tctaggaagc ctcgtacc 1348
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INTERNATIONAL SEARCH REPORT

	International Application No
	PCT/US 00/32378

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K38/17 A61K39/395 A61P37/00 A61P35/00 A61K31/00
A61K38/18 A61K38/19 C07K16/28 C07K16/46
//(A61K39/395,31:00),(A61K39/395,38:18)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

BIOSIS, EMBASE, WPI Data, PAJ, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	P. MOORE ET AL.: "BLyS: member of the tumor necrosis factor family and B cell stimulator." SCIENCE, vol. 285, 9 July 1999 (1999-07-09), pages 260-263, XP002142252 New York, NY, USA page 260, right-hand column, line 11 - line 14 page 263, left-hand column, line 2 - line 6 --- -/--	1,2,14, 17,18, 44-52,63

[X] Further documents are listed in the continuation of box C. [X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

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Date of the actual completion of the international search	Date of mailing of the international search report
17 April 2001	11/06/2001

Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Nooij, F

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	H. SHU ET AL.: "TALL-1 is a novel member of the TNF family that is down-regulated by mitogens." JOURNAL OF LEUKOCYTE BIOLOGY, vol. 65, May 1999 (1999-05), pages 680-683, XP000939092 New York, NY, USA page 682, left-hand column, line 19 - line 38 page 682, right-hand column, line 5 - line 12 figure 3	1,2,17, 18
X	M. HAHNE ET AL.: "APRIL, a new ligand of the tumor necrosis factor family, stimulates tumor cell growth." THE JOURNAL OF EXPERIMENTAL MEDICINE, vol. 188, no. 6, 21 September 1998 (1998-09-21), pages 1185-1190, XP002093077 New York, NY, USA page 1189, right-hand column, line 6 - line 11	19,20, 32,36, 37,54, 55,60,61
X	A. MUKHOPADHYAY ET AL.: "Identification and characterization of a novel cytokine, THANK, a TNF homologue that activates apoptoss, nuclear factor-kB and c-jun NH2-terminal kinase." THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 274, no. 23, 4 June 1999 (1999-06-04), pages 15978-15981, XP002142251 Baltimore, MD, USA abstract page 15978, right-hand column, line 58 -page 15979, left-hand column, line 3 page 15980, left-hand column, line 3 -right-hand column, line 11	1,2,14, 17,18,63
X	WO 99 12965 A (BIOGEN, INC.) 18 March 1999 (1999-03-18) claims 23-25,32-40	19,20, 32,36, 37, 54-62, 65-67

-/--

International Application No

PCT/US 00/32378

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	G. VON BÜLOW ET AL.: "NF-AT activation induced by a CAML-interacting member of the tumor necrosis factor receptor superfamily." SCIENCE, vol. 278, no. 5335, 3 October 1997 (1997-10-03), pages 138-141, XP002165404 New York, NY, USA page 138, right-hand column, line 35 - line 39 abstract	38-40
X	WO 98 39361 A (ST. JUDE CHILDREN'S RESEARCH HOSPITAL) 11 September 1998 (1998-09-11) page 56, line 32 -page 57, line 27 page 58, line 1 - line 28 claims 26-28,38,39	38-40
P,X	S. MARSTERS ET AL.: "Interaction of the TNF homologues BLyS and APRIL with the TNF receptor homologues BCMA and TACI." CURRENT BIOLOGY, vol. 10, no. 13, 29 June 2000 (2000-06-29), pages 785-788, XP000992744 London, GB the whole document	1-8,11, 15-26, 29,34-37
P,X	G. YU ET AL.: "APRIL and TALL-1 and receptors BCMA and TACI: System for regulating humoral immunity." NATURE IMMUNOLOGY, vol. 1, no. 3, September 2000 (2000-09), pages 252-256, XP000992743 New York, NY, USA the whole document	1-8,11, 15-26, 29,34-37

page 3 of 3

FURTHER INFORMATION CONTINUED FROM   PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-18, 44-53, 63, 64

     TALL-1 antagonists, compositions comprising them, and their (therapeutic) use.

2. Claims: 19-37, 54-62, 65-88

     APRIL antagonists, compositions comprising them, hybridomas producing them, and their (therapeutic) use.

3. Claims: 38-40

     Methods to use TACI agonists.

4. Claims: 41-43

     Methods to use BCMA agonists.

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9912965	A	18-03-1999	AU	9315298 A	29-03-1999
			AU	9316298 A	29-03-1999
			BR	9812433 A	26-09-2000
			BR	9812634 A	22-08-2000
			CN	1269832 T	11-10-2000
			CN	1270632 T	18-10-2000
			EP	1012270 A	28-06-2000
			EP	1027431 A	16-08-2000
			NO	20001240 A	10-05-2000
			NO	20001242 A	11-05-2000
			PL	339463 A	18-12-2000
			PL	339740 A	02-01-2001
			SK	3542000 A	14-08-2000
			WO	9912964 A	18-03-1999
WO 9839361	A	11-09-1998	US	5969102 A	19-10-1999
			AU	6685498 A	22-09-1998
			EP	0964874 A	22-12-1999